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Research Article

Young capitulum as important explant in *in vitro* mass propagation of gerbera (*Gerbera jamesonii*)

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Abstract

A new route of *in vitro* propagation of gerbera selected clones was successfully established using young capitula in tight buds and buds that were started to unfold stage as explant source. The one-fourth pieces of young capitula of tight flower stage and half-strength MS medium containing 0.25 mg/l BAP was the suitable for initiation and produced higher number of shoots per explant up to 3.8 shoots. The results were improved by culturing the one-fourth piece of 01.092 capitulums on MS medium fortified by 0.2 mg/l BAP and 0.02 mg/l NAA producing the highest shoot formation up to 8.5 shoots per explant with 28.7 leaves per explant and 2.1 cm leaf length. High multiple shoots were determined in third to fourth subculture periods and reduced thereafter with high multiplication rate noted on 01.092 clone. Shoots were easily rooted on half-strength MS medium supplemented with 2 g/l activated charcoal. Plantlets were transferred to *ex vitro* condition with 96.4% survivability of 03.045 clone using *Cycas rumphii* bulk and cocopeat (1:1, v/v) under spraying 1 g/l Growmore (32N:10P:10K) solution once week periodically. The route has high potential applied in qualified plantlet production for other Gerbera's due to high shoots produced up to 35 shoots per whole young capitulum used.

Keywords: acclimatization; capitulum; gerbera clones; initiation; media; regeneration

Introduction

Gerbera jamesonii (H. Bolus ex Bolus f.), both as cut flower or potted plants, is one of important flower commodities both in local and international market. Under protected cultivation technology, benefit cost ratio of Gerbera in Karnataka state of India could reach 5.25 (Gamanagatti and Patil, 2018), 2.94 in Chhindwara district of Madhya Pradesh (Deshmukh *et al.*, 2019); while 1.75 to 3.53 were noted in Indonesia (Agrowindo, 2015; Umkm-news, 2016). In Indonesia, total cultivation areas of the flower were 665.287 ha widespread at West, Central and East Java; Bali; and North Sulawesi provinces with 26.6 million stems of total production, 40 stems productivity per m^2 on 2017 (Badan Pusat Statistik dan Direktorat Jenderal Hortikultura, 2019a,b,c). Selling price was 10.000 to 40,000 rupiahs per bunch dependent on variety and quality (Izza-Florist, 2019; Tokopedia, 2019). Though the commodity has high economical value and market demand, development of Gerbera commercially is constrained by availability of qualified-planting materials sustainability.

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Conventionally, rhizome divisions and cuttings were applied for vegetative propagation of the gerbera. The vegetative method, though, can maintain uniformity and genetic purity, however it is laborious and time consuming with limited-results (Kanwar and Kumar, 2008). While seeds are generally utilized for generative propagation. The technique usually resulted in high number of regenerants with varied-planting materials genetically and phenotypically (Cardoso and Teixeira da Silva, 2013). Therefore, the both methods are not suitable applied in commercial scales. Whereas Mohamed and Ozzambak (2014) stated that large number of planting materials in uniform, vigorous and pathogen free performances that are produced in a shorter time with high sustainability are high potentially prepared by tissue culture works.

In vitro propagations of gerbera were successfully developed massively by so many researchers with different ways and results. The propagation was generally established using shoot tips, axillary buds, leaves, petioles, flower buds, flower stems, roots, petals, capitulums and seeds (Kanwar and Kumar 2008; Altaf *et al.*, 2009; Hasbullah *et al.*, 2015 and Vijayalakhmi *et al.*, 2019); Murashige and Skoog (MS, 1962) as culture medium (Nasari *et al.*, 2014; Niedz *et al.*, 2014; Winarto and Yufdy, 2017); via axillary proliferation (Naz *et al.*, 2012; Kadu, 2013; Winarto and Yufdy, 2017), adventitious shoot multiplication (Hussein *et al.*, 2008;) and embryogenesis (Ranjan and Guarav, 2005; Hasbullah *et al.*, 2011) either directly or indirectly applied in different varieties/cultivars (Shabanpour *et al.*, 2011; Son *et al.*, 2011). Each stage of the *in vitro* propagation of the flower is interesting and indicating different behavior responses specifically. Therefore, establishing of a new and reliable route of *in vitro* propagation of gerbera derived from young capitula is necessary.

Materials and Methods

Planting materials

Materials used in the study was selected clones of *G. jamesonii* of 01.092, 03.045 and 21.035 clones. The clones were cultivated under plastic house in Cipanas Experimental Garden, Landbow, Cipanas-Cianjur at 1100 m asl and maintained optimally via watering and fertigation periodically. Young un-opened and initial opened flower buds were harvested from the donor plants at 07.00 to 09.00 am (Figure 4A and 4B). The two types of explant were then brought to tissue culture laboratory of Indonesian Ornamental Crop Research Institute for sterilization purposes.

Explant sterilization and preparation

Sterilization of explants was carried out by shortening inflorescence length, pre-treating under running tap water for 60 min, immersing in 1% soap solution and shaking manually for 30 min, 1% pesticide solution (50% benomil and 20% kanamycin sulphate) for 30 min and rinsing 4-5 times (@ 3 min each) using distilled water to remove all remain disinfection materials. In the laminar air flow cabinet, they were soaked in 0.05% mercury chloride ($HgCl_2$) solution supplemented with 5 drops of Tween 20 for 5 min, then rinsing with sterile distilled water (SDW) 4-6 times (@ 3 min each). Furthermore, all sepals and petals were removed carefully using small pinset till all capitulum of three clones clear and free from them, then sliced vertically into four pieces (Figure 7C), soaked in 25 ml Erlenmeyer containing 10 ml SDW and further sterilized by immersing them in 0.01% $HgCl_2$ solution for 3 minutes. Finally, the explants were rinsed by SDW 4-6 times (@ 3 min each). The ¼ portion of sliced capitulum were then cultured on the initiation media (Figure 4D).

Culture incubation

Cultures of explants were incubated in the dark for ± 30 days for initial shoot regeneration; thereafter, the shoots were then placed under light incubation of cool fluorescent lamps with ~13.5 µmol/m/s light intensity, 12-h photoperiod and 23.5 ± 1.1 °C till shoots regenerated. The light incubation was also applied for multiplication and root formation.

Studying different young capitulum stages and initiation media on shoot regeneration

Two stages of young capitula of 03.045 clone namely (1) tight bud stage and (2) buds that were started to unfold were studied in the experiment. Initiation media (IM) tested in the experiment were (1) MS medium supplemented with 2.0 mg/l BAP and 0.2 mg/l NAA (IM-1, control); (2) half-strength MS medium containing 2.0 mg/l TDZ and 0.05 mg/l BAP (IM-2), (3) 1.5 mg/l TDZ and 0.25 mg/l BAP (IM-3), (4) 1.0 mg/l TDZ and 0.25 mg/l BAP (IM-4), (5) 0.5 mg/l TDZ and 0.25 mg/l BAP (IM-5), and (6) 0.25 mg/l BAP (IM-6). All media fortified by 3% sucrose and 7 g/l agar Swallow. Media pH was adjusted to 5.8 and autoclaved at 121 °C and a pressure of 1.05 kg cm^{-2} for 20 min. A factorial experiment was arranged in a randomized complete block design with 3 replications. Each experimental unit consisted of 4 pieces of young capitulum.

Response of three selected clones cultured in different initiation media on shoot formation

Explants used in the experiment were one-fourth pieces of young capitula of 01.092, 03.045 and 21.035 clones. While initiation media (IM) tested in the experiment were half-strength MS medium containing 0.2 mg/l TDZ (IM-1), 0.2 mg/l TDZ and 0.10 mg/l BAP (IM-2); MS medium supplemented with 0.2 mg/l TDZ (IM-3), 0.2 mg/l TDZ and 0.1 mg/l BAP (IM-4),0.2 mg/l BAP and 0.02 mg/l NAA (IM-5, as control), and 0.2 mg/l TDZ and 0.02 mg/l NAA (IM-6). All media were prepared as previously mentioned. The experiment was arranged in split plot design with four replications. Each treatment consisted of 3 bottles. Each bottle contained 4 pieces of explant.

Variables observed in the first and second experiments were (1) percentage of explant regeneration (%), (2) number of initial shoots per explant, (3) number of shoots per explant; (4) number of leaves per explant and (5) length of leaves (cm). Periodical observation was carried out to know response of explants during incubation period. Variables were recorded and measured 1.9-2.1 months after culture

Shoot multiplication study

To multiply shoots, single shoots of 03.045, 21.045 and 01.092 clones were subcultured periodically on MS medium fortified with 0.2 mg/l BAP and 0.02 mg/l NAA till peak shoot production established and declined thereafter. Each treatment consisted of 10 bottles. Each bottle contained 5 single shoots. Variables observed were (1) number of shoots per single shoot cultured, (2) total number of leaves and (3) length of leaves (cm). Variables were recorded and measured 1.5-1.6 months after culture.

Plantlet acclimatization study

Rooted shoots sufficiently were easily obtained by culturing 1-2 shoots established previously on half-strength MS medium containing 2 g/l activated charcoal (AC). Each culture vessel consisted of 5 explants. The cultures were incubated under light incubation for 1.5-1.6 months. Healthy rooted shoots with 2-6 roots per shoot and 0.3 to 0.6 cm root length were then taken gently from culture vessels, washed under tap water to remove remaining agar from root surface, immersing in 1% pesticide solution of fungicide and bactericide for ± 3 minutes (Figure 4O) and drying for a while on paper. After the treatment, plantlets were planted on plastic tray (30 × 15 × 10 cm; length × width × height) containing acclimatization media with ± 6 cm in thickness, covered with transparent plastic for 30 days and placed on low light intensity area (Figure 4P).

In the stage, three different treatments i.e. periodical spraying liquid compound fertilizer, acclimatization media and responses of clones were studied gradually. The periodical spraying and un-spraying of 1 g/l Growmore (32P:10N:10K) solution once a week in the morning were applied 7 days after acclimatization. The acclimatization media tested were commercial media; burned rice husk and organic manure (1:1, v/v); and *Cycas rumphii* bulk and cocopeat (1:1, v/v). The three clones tested were 01.092, 03.045 and 21.035 clones. The experiments were conducted serially and arranged in a completely randomized design with enough replications for each treatment. Variables observed in the study were survival percentage of plantlets and number of plantlets successfully established. All variables were recorded and measured 1.9-2.1 months after acclimatization.

Data analysis

Quantitative data in all experiments were analysed by analysis of variance (Anova). Significant differences between means were assessed by Tukey test at $P = 0.05$ (Mattjik and Sumertajaya, 2006).

Results and Discussion

These experiments clearly revealed that initial shoots frequently accompanied by callus formation were obviously observed 18-33 days after culture initiation in dark incubation (Figure 4E). Browning explants due to reaction between phenolic compounds produced by explants and medium components also occurred with 0-25% from total explants cultured. The shoots continually grew and produced leaves after transferring them under light incubation with 10-100% percentage of explant regeneration and 1-25 initial shoots per explant. After 1,5 months under light incubation, number of shoots per explant were 1-15 shoots (Figure 4F) with 10-33 leaves per explant and 0.5 - 3.0 cm length of leaves.

Studying different capitulum stages and initiation media on shoot regeneration

The one-fourth pieces of young capitula and initiation media gave statistically significant effects on initiation and regeneration of shoots. Young capitulum from tight bud stages gave higher effects compared to those started to unfold. The one-fourth pieces of the capitulum stimulated initial shoots in a shorter period with lower percentage of browning explant, higher percentage of explant regeneration, 7.8 initial shoots per explant and 1.2 shoots per explant (Figure 1A). While the best initiation medium was recorded on IM-6 medium (half-strength MS medium containing 0.25 mg/l BAP). Though the medium induced lower results in most of variables, however the treatment successfully produced high shoot regeneration up to 2.9 shoots per explant (Figure 1B).

The one-fourth pieces of young capitulum from tight bud stages cultured on IM-6 medium was the best combination treatment for shoot regeneration. Though the combination gave lower results in the most variables (Supplement data 1, 2 and 3 not shown; Table 1), however the treatments produced higher number of shoots per explant up to 3.8 shoots (Table 2). The second-best treatment was performed by the young capitulum from those buds started to unfold in the same medium. While other combination treatments, though resulted in higher data in the most variables observed, they stimulated lower number of shoots per explant than the previous combinations.

Table 1. Interaction effect of different young capitulum stages and initiation media on number of initial shoots produced per explant

One-fourth pieces of young capitulum stage	Initiation media (IM)						Means
	IM-1	IM-2	IM-3	IM-4	IM-5	IM-6	
Tight flower buds	1.3 a	7.5 a	8.0 a	9.0 a	15.5 a	5.5 a	7.80
Flower buds that started to unfold	1.0 a	4.0 b	4.5 b	6.3 b	10.5 b	5.6 b	5.32
Means	1.20	5.75	6.25	7.65	13.0	5.55	
Coefficient of variation (CV, %)	23.28	13.32	19.11	11.31	18.78	13.47	

Means followed by the same letter in the same column are not significantly different based on Tukey test, $p=0.05$.

Table 2. Interaction effect of different explant types and initiation media on number of shoots produced per explant

One-fourth young capitulum	Initiation media (IM)						Means
	IM-1	IM-2	IM-3	IM-4	IM-5	IM-6	
Tight flower buds	0.8 a	1.5 a	0.8 a	0.5 a	1.0 a	3.8 a	1.40
Flower buds that started to unfold	0.5 a	1.0 a	0.0 b	0.3 a	1.0 a	2.0 b	0.80
Means	0.65	1.25	0.40	0.40	1.00	2.90	
Coefficient of variation (CV, %)	17.76	21.59	19.36	12.67	16.79	11.73	

Means followed by the same letter in the same column are not significantly different based on Tukey test, *p=0.05*.

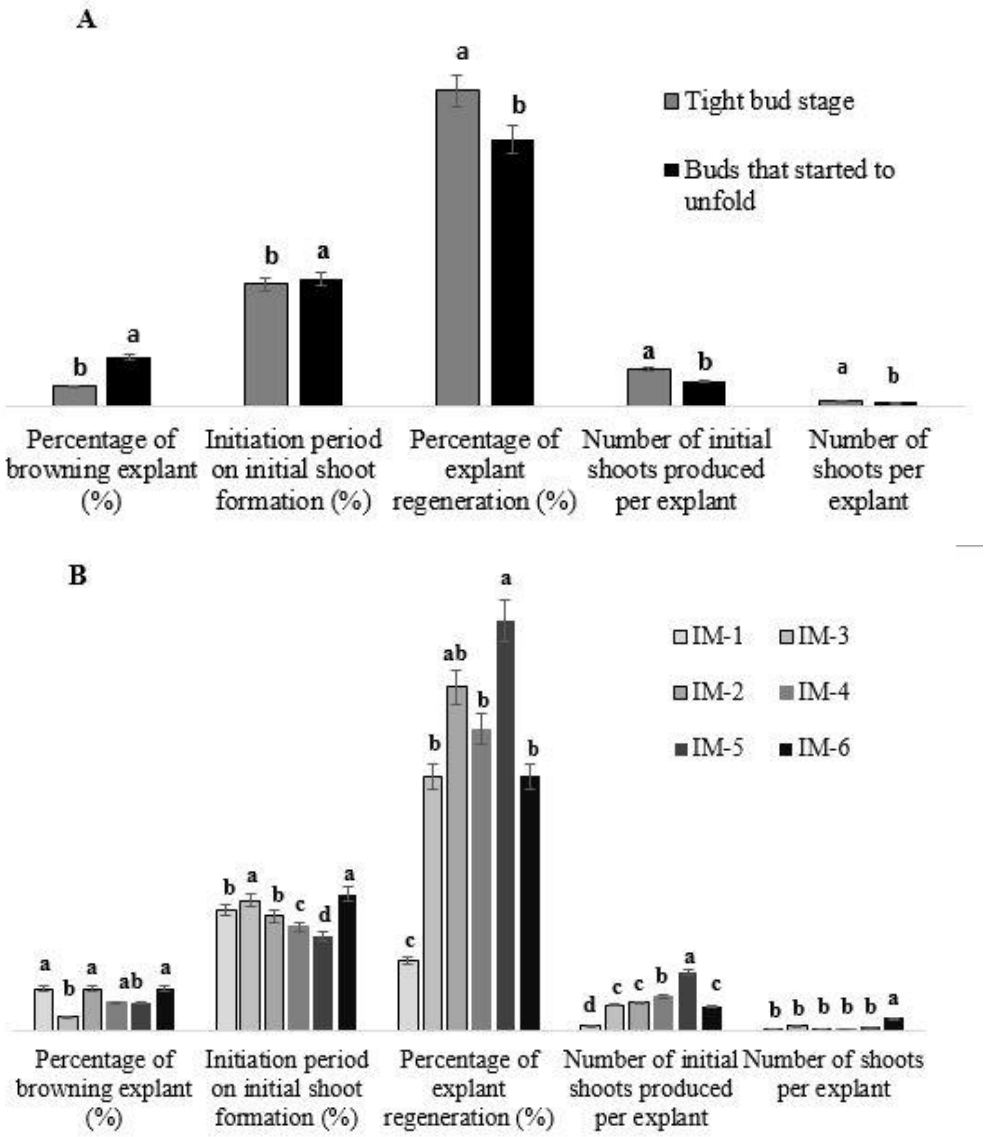

Figure 1. Effect of different stages of flower bud and initiation media on initiation and regeneration of shoots. A. Responses of different explant types. B. Effect of different initiation media. Histograms followed by the same letter in the same cluster are not significantly different based on Tukey test, p=0.05

Response of three selected clones cultured in different initiation medium on shoot formation

There were different responses of the three selected clones cultured on different initiation media in all variables observed. Both treatments gave significant and interaction effect on the shoot formation, in which the selected clones stimulated higher effect than the initiation media. Furthermore, 01.092 clone was the most responsive clone in inducing higher shoot formation. The clone successfully produced 5.6 shoots per one-fourth explant with 21 leaves per explant and 1.90 cm in leaf length (Figure 2A). While IM-5, MS medium augmented with 0.2 mg/l BAP and 0.02 mg/l NAA, was the most suitable medium for inducing shoot formation up to 6.1 shoots per explant with 22.6 leaves per explant and 1.75 cm in length of leaves (Figure 2B). The second-best treatment was performed by IM-2 and IM-4 with the lowest results noted by IM-1.

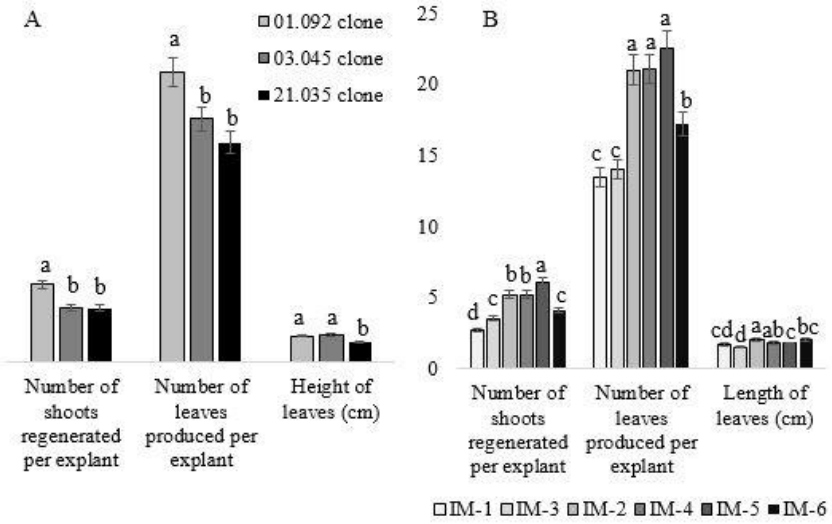

Figure 2. Responses of selected gerbera clone and initiation media on shoot formation. A. Responses of selected gerbera clone. B. Effect of initiation media. Histograms followed by the same letter in the same histogram cluster are not significantly different based on Tukey test, *p=0.05*

The best combination treatment was recorded on the young capitulum of 01.092 clone cultured on IM-5 medium. The combination successfully regenerated 8.5 shoots per one-fourth explant (Table 3) with 28.7 leaves per explant (Table 4) and 2.08 cm leaf length (Supplement data 4 not shown). The second-best combination was exhibited by culturing the same explant on IM-4 medium. The medium also resulted in higher number of shoots for 21.035 clone, while for 03.045 clone, the optimal shoot formation was noted on IM-2. The results strengthened and gave evident that each genotype had different responses and behaviors in *in vitro* culture performances.

Table 3. Interaction effect of clones and media on number of shoots per explant

Medium	Gerbera selected clones			Means
	01.092	03.045	21.035	
IM-1	2.8 c	1.9 d	3.4 bc	2.70
IM-2	3.5 c	3.0 cd	4.0 ab	3.50
IM-3	5.5 b	7.0 a	3.2 bc	5.23
IM-4	7.9 a	2.9 cd	4.9 a	5.23
IM-5	8.5 a	5.0 b	5.0 a	3.08
IM-6	5.8 b	3.8 bc	2.3 c	3.97
Means	5.67	3.93	3.80	
Coefficient of variation (CV, %)	8.94	10.03	10.39	

Means followed by the same letter in the same column are not significant different based on Tukey test, *p=0.05*.

Table 4. Interaction effect of clones and media on number of leaves per explant

Media	Gerbera selected clones			Means
	01.092	03.045	21.035	
IM-1	14.3 bc	11.0 c	15.4 abc	13.57
IM-2	13.8 c	14.3 bc	13.9 bc	14.00
IM-3	20.5 b	26.5 a	16.0 abc	21.00
IM-4	28.3 a	17.0 bc	18.1 ab	21.13
IM-5	28.7 a	20.4 b	18.6 a	22.57
IM-6	20.8 bc	16.8 bc	12.8 c	16.80
Means	21.07	17.67	15.80	
Coefficient of variation (CV, %)	10.32	11.03	8.06	

Means followed by the same letter in the same column are not significant different based on Tukey test, $p=0.05$.

Periodical subculture of shoots in the medium containing TDZ resulted in malformation of shoots, especially after the third subculture. The malformation of shoots was significantly noted in IM-2 and IM-4 with indicating wavy and stunted-leaves and developing callus in the basal petiole (Figure 4K, 4L and 4M). The such shoots were not suitable for preparing qualified shoots; therefore, the medium was not used for shoot multiplication. The best shoot performances were noted from young capitulum cultured on IM-5 medium. The IM-5 medium could also be used to recover the malformation of shoots for resulting good quality shoot performances.

Shoot multiplication study

Study on shoot multiplication carried out by culturing single shoot on the selected medium resulting in interesting performances in all variables observed (Figure 4G and 4I). In clone of 01.092, number of regenerated shoots, leaves and leaf length produced after incubation increased gradually in first (Figure 4H), second till third subculture and reduced thereafter (Figure 3A and 4B). Second best results were performed by 03.045 clone. While for 21.035 clone, the gradual increasing of the variables was extended till the fourth subculture. Number of regenerated shoots of 01.092 clone from 5.3 shoots in the first subculture enhanced to be 6.9 in the second subculture and 8.5 shoots in the third subculture (Figure 4J), then decreased down to 7.2 shoots in the fourth and 6.2 in the fifth subculture. Similar performances with different values were recorded on number of leaves per explant and leaf length. The value alteration was also recorded on 03.045 and 21.035 clones.

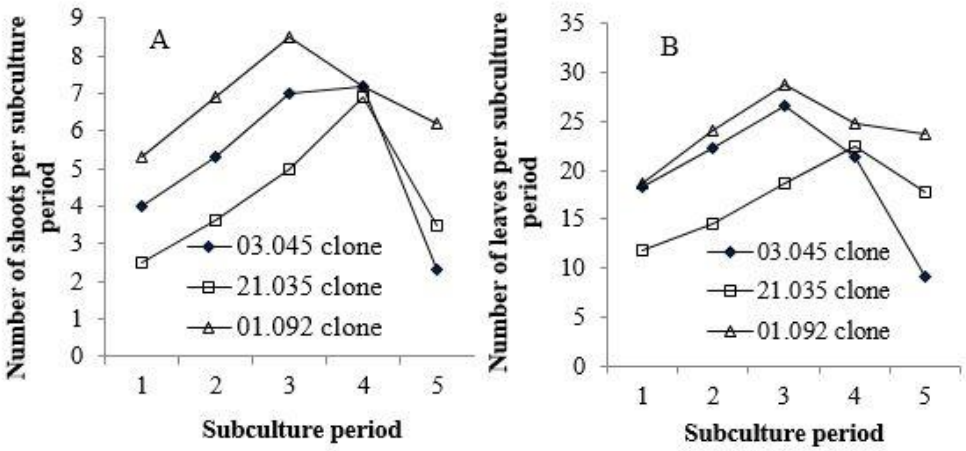

Figure 3. Shoot multiplication of the selected clones under periodical subculture. A. Number of shoots per subculture period, B. Leaf number per subculture period

Plantlet acclimatization study

Though plantlet acclimatization was studied previously, significant results in the acclimatization were performed in the research. The results were stimulated by spraying 1% Growmore (32N:10P:10K) solution periodically once week. The treatment successfully induced survival plantlets up to 88-100% with 95.3% in average with 47.5 plants in good quality performances (Table 5). Furthermore, acclimatization medium of *C. rumphii* bulk and cocopeat (1:1, v/v) was the optimal medium for plantlet acclimatization with 95% survivability and 47.5 survival plantlets (Table 6). Whereas plantlets of 03.045 clone were the easiest plantlets handled in the acclimatization stage with 96.4% survivability and 48.2 survival plantlets (Table 7; Figure 4Q). Acclimatized plants grew optimally after repotting individually (Figure 4R).

Table 5. Effect of spraying Grow more (32N:10P:10K) solution on Gerbera plantlet acclimatization

Treatment	Percentage of plantlet survivability (%)	Number of survivals plantlets
No spraying Growmore solution	12,5 b	6,3 b
Spraying Growmore solution	95,0 a	47,5 a
Coefficient of Variation (CV, %)	10.12	10.67

Means followed by the same letter in the same column are not significant different based on Tukey test, *p=0.05*.

Table 6. Effect of acclimatization media on Gerbera acclimatization plantlets

Type of media	Percentage of plantlet survivability (%)	Number of survival plantlets
Commercial medium	*48,5 b*	*24,3 b*
Burned rice-husk and organic manure (1:1, v/v)	71,0 ab	35,5 ab
Cycas rumphii bulk and cocopeat (1:1, v/v)	95,0 a	47,5 a
Coefficient of Variation (CV, %)	11.22	12.05

Means followed by the same letter in the same column are not significant different based on Tukey test, *p=0.05*.

Table 7. Responses of Gerbera clones on acclimatization

Gerbera clone	Percentage of plantlet survivability (%)	Number of survival plantlets
21.035	78,0 b	39,0 b
03.045	96,4 a	48,2 a
14.028	71,6 b	35,8 b
Coefficient variation (CV, %)	10.35	10.83

Means followed by the same letter in the same column are not significant different based on Tukey test, *p=0.05*.

Entirely the research was successfully established a new route of *in vitro* mass propagation of gerbera using young capitulum as explant source. Interesting evident were revealed in the initiation and regeneration of shoots. High callus and number initial shoots regenerated under high concentration-combination of TDZ, BAP and NAA that were suitable for *in vitro* mass propagation of gerbera using shoot tip under axillary proliferation (Winarto and Yufdy, 2017) were not appropriate for young capitulum. In the study, high shoot regeneration was established on half-strength MS medium supplemented with 0.25 mg/l BAP as coincidence with the previous study under tTCL treatment of shoot tips (Winarto *et al.*, 2019). The experiment was significantly improved by culturing the one-fourth of young capitulum from tight flower buds on MS medium containing 0,2 mg/l BAP and 0.02 mg/l NAA and resulted in 8.5 shoots per explant with 28.7 leaves per explant and 2.08 cm leaf length. So, compilation of four pieces of young capitulum had high potential on shoot regeneration compared to culturing whole young capitulum without slicing. From four pieces of one-fourth of young capitulum could produce 25-35 shoots, while from the whole young capitulum could only stimulate 10-

15 shoots. The higher shoot regeneration capacity was presumably induced by higher potential of one-fourth explants in nutrition absorption due to increasing slicing effect.

Figure 4. A new route of *in vitro* propagation protocol of gerbera using young capitulum as explant source. A. Tight flower bud, B. Flower buds that were started to unfold, C. Four pieces of young capitulums ready cultured on initiation media, D. Four pieces of explants cultured on initiation media in initial culture, E. Responsive young capitulum of 01.092 clone on MS medium containing 0.2 mg/l BAP and 0.02 mg/l NAA 30 days after dark incubation, F. High number of shoots from one-fourth piece of 01.092 clone on similar medium 1.5 months after light incubation. G. Isolated shoots from initial culture for shoot multiplication. H. Multiple shoots of 01.092 clone in second subculture 30 days after subculture, I. Single shoots subculture for multiplication purpose, J. Multiple shoots in the third subculture, K-M. Malformation of shoots under TDZ containing media. N. Rooted shoots of 03.045 clone in half-strength MS medium containing 2 g/l AC ready for acclimatization, O. Rooted shoots immersed in 1% pesticide solution for 3 minutes, P. Plantlets cultured in plastic tray containing *C. rumphii* bulk and cocopeat (1:1, v/v) ready covered by transparent plastic for 30 days, Q. Healthy and vigor performances of acclimatized-plantlets cultured on *C. rumphii* bulk and cocopeat (1:1, v/v) 1.5 months after acclimatization, R. Individual plant of 03.045 clone 3 months after acclimatization

Bhatia *et al.* (2012) established 10.4 shoots per explant using the immature capitulum of *G. jamesonii* 'Cabana' cultured on MS medium supplemented with 10 mg/l BAP and 1 mg/l NAA. MS medium containing 6 mg/l BAP and 2 mg/l Kinetin was applied to produce 11.1 shoots per explant of *G. jamesonii* 'Harley' immature capitulum (Bhargava *et al.*, 2013). MS medium augmented with 2.0 mg/l BAP and 1.0 mg/l IAA was used to regenerate 10.5 shoots per explant with 4.8 leaves per explant of *G. jamesonii* 'Panama' (Mishra *et al.*, 2014), and MS medium fortified by 2.0 mg/l BAP and 0.5 mg/l NAA was applied to induce 25 shoots per culture (Swetha *et al.*, 2017). From the previous studies and the research, there were evident that younger capitulums gave better response in the shoot initiation and regeneration than that of the older one (Bhatia *et al.*, 2012).

Each explant/genotype/variety having specific response and behaviour in *in vitro* culture of gerbera was also revealed in the study. The specific response and behaviour were determined in all stages of micropropagation from initiation, regeneration, proliferation till acclimatization. In the most cases, 01.092 clone induced higher results in initiation, regeneration and proliferation than 03.045 and 21.035 clones, but in acclimatization, 03.045 clone showed better results than others. In other studies, it was reported that young capitulum of *G. jamesonii* 'Panama' gave better results on shoot regeneration than that of *G. jamesonii* 'Tamara' (Mishra *et al.*, 2014). *G. jamesonii* 'Antibes' exhibited better results on sterilization process, callus formation, shoot regeneration and plantlet growth derived from young capitulum than *G. jamesonii* 'Roll Roills' (González and Niño, 2014).

Periodical subcultures of shoots derived from the initiation stage were generally applied to explore their capacity of shoots in regenerating new shoots till their peak production and declined thereafter. In the study 01.092 clone was better than 03.045 and 12.035 clones on multiplication of shoots. Shoots increased gradually from first, second and third subculture and/or fourth subculture then decreased afterwards. Whereas other variables of leaves number and leaf length, the three clones had almost similar trend. The results were almost similar with subculturing shoots derived from tTCL shoot tips of varied varieties and clones (Winarto *et al.*, 2019), but it was lower than subculturing axillary shoots derived from shoot tips (Winarto and Yufdy, 2017).

Interesting phenomenon was noted from the study, especially immersing abnormality of shoots after periodical subculture of shoots on TDZ containing media. The abnormality was determined on media with 0.2 mg/l TDZ individually or/in combination with BAP after the third subculture. The new shoots having wavy leaves, stunted shoots and high callus formation in the basal part of leaf petioles indicated high sensitivity of new shoots on the existence of TDZ hormone. According to Vinoth and Ravindhran (2018) supra-optimal level of TDZ produces shoot abnormalities such as stunted shoots or fused shoots, while prolonged exposure to TDZ resulted in callus necrosis or reversal of shoot buds. In other studies, application of 1.0 mg/l TDZ for *in vitro* cultures of *Musa acuminata* 'Berangan Intan' and 'Berangan', and 0.5 mg/l TDZ for 'Baka Baling', 'Nangka' and 'Rastali' resulted in high number of abnormal shoots (Shirani *et al.*, 2009). TDZ in 2 mg/l and 0.5 mg/l NAA produced high levels of stunted shoots, while 3 mg/l TDZ and 0.5 mg/l NAA produced malformation of shoots in *in vitro* culture of *Aloe vera* (Lavakumaran and Seran, 2014), 0.2 mg/l TDZ increased morphological abnormal plants of dwarf plants of *Musa* spp 'Rajapuri' (Manjula *et al.*, 2014).

Significant healthy and vigour growth performances of plantlets, in the study, were established under spraying 1 g/l Growmore once week and using *C. rumphii* bulk and cocopeat (1:1, v/v) as acclimatization media. The treatment resulted in high survivability of plantlets up to 96.4% and noted on 03.045 clone. The study improved quality performances of plantlet growth compared to the previous studies (Winarto and Yufdy, 2017; Winarto *et al.*, 2019). Bhatia *et al.* (2012) established acclimatization of *G. jamesonii* 'Cabana' plantlets up to 93% using sterilized peat and soil rite mixture saturated with ½ strength MS medium containing only macro- and micro-salts using glass jars with polypropylene caps. Cocopeat in plastic pots and covering plantlets with polythene for 4 weeks induced survivability of *G. jamesonii* 'Harley' plantlets up to 90% (Bhargava *et al.*,

2013), vermiculite + vermicompost (1:1, v/v) medium was utilized to get high percentage of survival rate of plantlets up to 80% (Swetha *et al.*, 2017).

Conclusions

Finally, it can be concluded that a new route of the *in vitro* propagation of gerbera using young capitulum was successfully established. In initial step, young capitulum from un-opened flower buds and half-strength MS medium containing 0.25 mg/l BAP was suitable explant and medium for initiation and regeneration shoots. The results were improved by culturing the ¼ explant of 01.092 clone on MS medium with 0.2 mg/l BAP and 0.02 mg/l NAA to obtain the highest shoot formation. Though the peak production of shoots of 01.092, 03.45 and 21.035 clones were determined in the third to fourth subculture periods, the high response of shoot multiplication was noted on 01.092 clone. Shoots were easily rooted on half-strength MS medium fortified by 2 g/l AC. The rooted shoots were successfully transferred to *ex vitro* condition with high survivability up to 96.4% on 03.045 clone using *C. rumphii* bulk and cocopeat (1:1, v/v) under spraying 1 g/l Growmore solution once week periodically.

Authors' Contributions

B.W. contributed to research planning and all intermittent steps until manuscript preparation and revisions. Y.K. was involved in preparing research materials. While R.H. was joined in helping data analysis, experimental design, and manuscript writing and editing. All authors approved the manuscript for publication, take public responsibility for the content.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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