academicJournals

Vol. 15(44), pp. 2497-2506, 2 November, 2016
DOI: 10.5897/AJB2016.15639
Article Number: 9CA266E61404 x
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

In vitro direct organogenesis in response to floral reversion in lily

Asker H. M.

Unit of Plant propagation, University of Baghdad, Baghdad, Iraq.

Received 31 August 2016; Accepted 18 October, 2016

Our previous study indicated that the tiger lily (*Lilium lancifolium* var. Flore Pleno) has a great ability to produce inflorescence bulbils in nature as a form of natural phenomenon of floral reversion in plants. This present research was carried out to investigate the artificial floral reversion in *in vitro* culture of two lilies (Asiatic hybrid cv."Black out"), and (*Lilium longiflorum* cv "White heaven") based on the type and developmental stage of explants plus the different concentrations of naphthalene acetic acid (NAA) and benzyl aminopurine (BA). Developmental changes were observed in both lilies in response to floral reversion which was enhanced by growth regulators under *in vitro* condition. The regeneration of vegetative organs was associated with certain degeneration of floral organs. Large bulblets and multiple shoots were formed only in specific regions in floral organs, precisely in two attached points: the boundary region between the receptacle with other floral organs and the branching point where the peduncle joins the pedicel. This direct organogenesis was highly dependent on type of lily, type and developmental stage of explants in addition to the concentration of BA and NAA in *in vitro* culture. However, 1 mg/L BA combined with 0.1 mg/L NAA was the optimum for regenerating shoots and bulblets in *in vitro* culture of both lilies after six weeks.

Key words: Floral reversion, organogenesis, lilies, active points, growth regulators, *in vitro*, bulblets.

INTRODUCTION

In nature, there are more than 250,000 species of flowering plants, and they represent the most wide spread groups of plants. Flowers are important sexual reproductive organs of flowering plants and source of fruit and seed for completing plants' life cycle.

However, in some species, the phenomenon of floral reversion occurs rarely in nature in response to adverse environment and it is affected by photoperiod and hormones. Furthermore, this phenomenon can be efficiently induced in some species *in vitro* under optimal concentrations of auxins and cytokinins (Tooke et al., 2005; lashman and Kamenetsky, 2006; Supriyo et al., 2013). It can also be an excellent method for propagating some ornamental geophytes. It has been shown that it is a great alternative to explants of underground storage organ for overcoming the problem of heavy contamination which usually occurs in these organs (Ziv and Lilien- Kipnis, 2000; Poluboyarova et al., 2011)

E-mail: Hassan.asker2@gmail.com.

Various floral organs such as pedicel and filament were taken from some geophytes as explants to regenerate shoots and bulblets in *in vitro* culture (Kumar et al., 2006; Nhut et al., 2001). The study of Liu and Burger (1986) observed that the explants which were taken very close from the receptacle and the most distilled section of the pedicel produced the greatest number of buds in *in vitro* culture (*Lilium longiflorum*).

In recent years, the histological study of *Allium altissimum* (Poluboyarova et al., 2014) showed that the morphogenic tissue in the fused area of stamens and sepals had the potency to regenerate shoot in *in vitro* culture. Moreover, several molecular genetic studies confirmed that the pedicel parts are different in their developmental processes in the main model plant (*Arabidopsis thaliana*) (Douglas and Riggs, 2005) and are genetically regulated by several genes (Cho and Cosgrove, 2000; Kirik et al., 1998; Song and Clark, 2005; Ragni et al., 2008).

The objective of the current study was to investigate the artificial floral reversion of two lilies based on the type and developmental stage of explants in addition to the concentrations of naphthalene acetic acid (NAA) and benzyl aminopurine (BA) in *in vitro* culture.

MATERIALS AND METHODS

This study was designed to investigate the organogenetic response of two lilies, Asiatic hybrid cv."Black out" and *L. longiflorum* cv "White heaven" to floral reversion process in *in vitro* culture. Three cultural experiments were done *in vitro*. The inflorescence segments were collected from plants grown in computerized greenhouses at the school of biological sciences, University of Plymouth during the year 2014. The explants were carefully washed and sterilized with 10% v:v sodium hypochlorite for 15 min. They were washed three to four times with sterilized distilled water before culturing. The explants were then cultured on Murashige and Skoog (MS) basal medium containing (30 g L^{-1}) sucrose (8 g L^{-1}) agar, pH 5.7, supplemented with different concentrations of NAA and BA. All cultures were incubated in a Gallenkamp growth cabinet under 16 h photoperiod, provided by cool-white fluorescent lamps with an irradiance of 100 µmol m^{-2} s^{-1} at a constant temperature of (25°C).

Three types of explants were taken for this study: explants of receptacle, explants of the branching point where the peduncle joins the pedicel and explants of whole flower bud. Four concentrations of BA and NAA: (1 mg/L BA+1 mg NAA), (1 mg/L BA+0.5 mg NAA), (1 mg/L BA+0.1 mg NAA) and (0 mg/L BA+0 mg NAA) as control were used. Two different developmental stages of explants were chosen: young explants were taken when the size of floral bud was 4 to 6 cm and mature explants were taken at fully mature stage. The receptacle explants were prepared by cutting the portion of receptacle into two identical pieces and the half piece was cultured horizontally on agar ; otherwise, all other explants were placed vertically on the agar.

Each experimental treatment was carried out with at least 15 explants per treatment. The experiment was arranged in a completely randomized block design. The number and weight (g) of bulblets and roots per explant, the number, weight (g) and length (cm) of shoots and the weight of ovary per explant were recorded after six weeks of culture *in vitro.*

The statistical analysis SAS system (SAS, 2012) was used to show the effect of different factors on the study parameters. Significant difference (LSD) test was used in this study to compare between means at the 0.05 level of significance.

RESULTS

Plate 1 (for Asiatic hybrid lily cv. Black out) and Plate 2 (for *L. longiflorum* cv. White heaven) shows that the processes of floral reversion after six weeks in *in vitro* culture, converting floral to vegetative organogenesis resulted in the formation of a wide range of vegetative organs using young and mature receptacle explants with different concentrations of naphthalene acetic acid (NAA) and benzyl aminopurine (BA) (Plates 1 and 2).

Plate 3 records the *in vitro* culture of Asiatic hybrid lily after six weeks using whole floral buds explants with different concentrations of (NAA) and (BA). The bulblets and shoots appeared on specific points in floral organs: receptacle boundary and in branching points of pedicel-peduncle of explants. Plate 4 shows the *in vitro* culture of Asiatic hybrid lily using young and mature branch explants of pedicel- peduncle with constant concentration of 1 mg/L BA combined with 0.1 mg/l NAA. Plate 5 shows the *in vitro* culture of longiflorum lily after six weeks using mature whole floral bud explants and mature receptacle explants with constant concentration of 1 mg/L BA combined with 0.1 mg/L NAA. Figure 1 records the results of the *in vitro* cultures of Asiatic hybrid lily for six weeks using different type and developmental stage of explants plus different concentrations of NAA and BA.

The explants of inflorescence stalk branch have higher ability to regenerate bulblets and shoots than receptacle ones (Figure 1A). The young explants produced more shoots but less bulblets compared to the mature ones (Figure 1B). 1 mg/L BA combined with 0.1 mg/L NAA was the optimum for regeneration of shoots and bulblets compared to others (Figure 1C). The interaction effect of all these experimental factors is indicated in Figure 1D and the results confirm that the regeneration ability of explants is dependent on all these factors. Figure 2 shows the results of *in vitro* cultures of both lilies for six weeks using different concentrations of NAA and BA with young receptacle explants. Longiflorum lily has higher ability to regenerate bulblets and shoots than Asiatic hybrid lily (Figure 2A). The interaction effect between the type of lily and concentrations of NAA and BA is recorded in Figure 2B, and the results confirm that the regeneration ability of explants is clearly dependent on these experimental factors.

Figure 3 records the results of the experiment of *in vitro* cultures of both lilies for six weeks using different type and developmental stage of explants with constant concentration of 1 mg/L BA combined with 0.1 mg/L NAA.

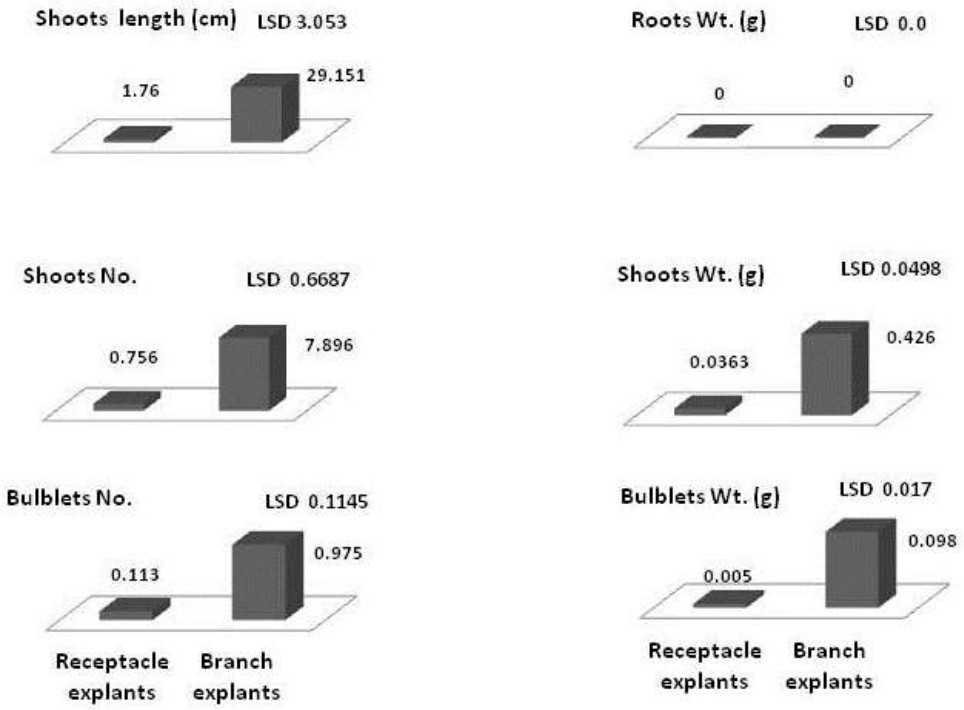

Figure 1A. Regeneration ability of explants of Asiatic hybrid lily after six weeks of culture *in vitro* as influenced by the type of explants. Using LSD test (p≤0.05).

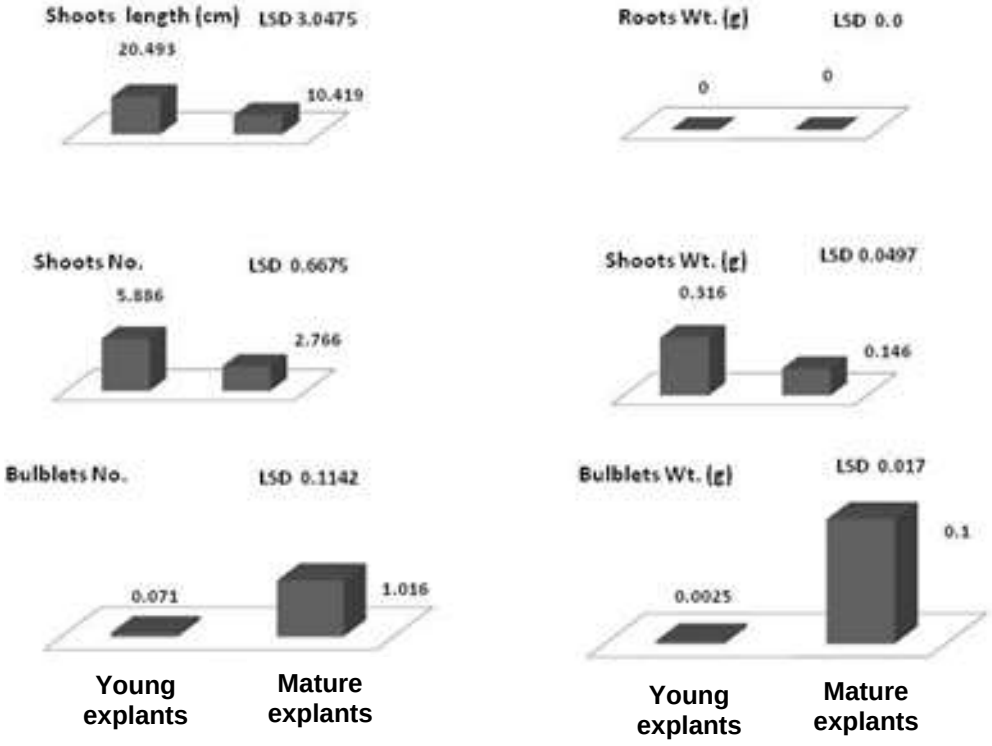

Figure 1B. Regeneration ability of explants of Asiatic hybrid lily after six weeks of culture *in vitro* as influenced by the developmental stage of explants. Using LSD test (p≤0.05).

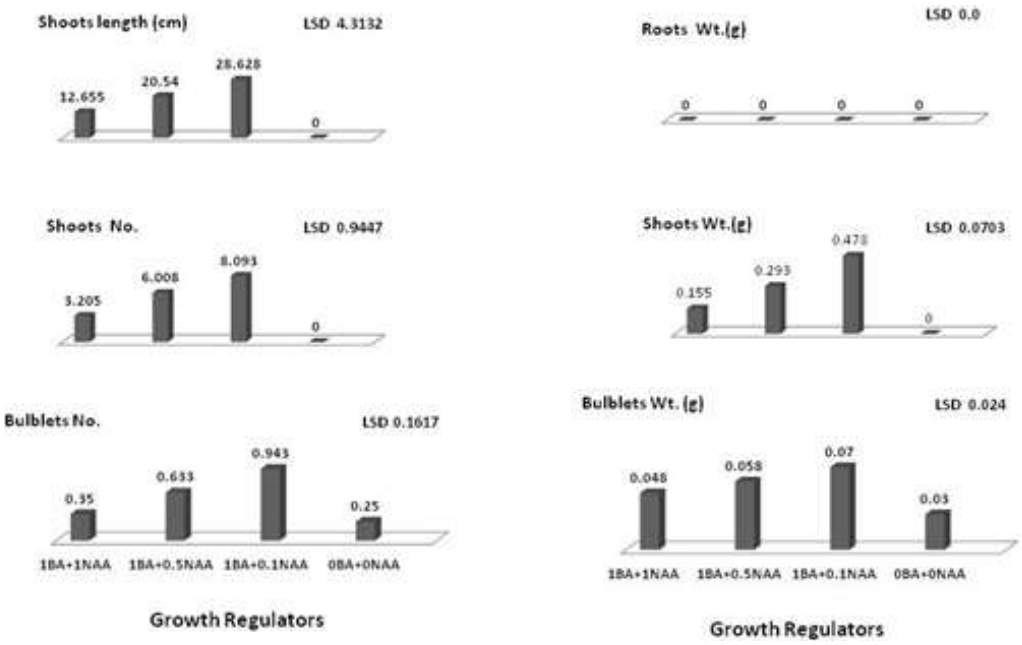

Figure 1C. Regeneration ability of explants of Asiatic hybrid lily after six weeks of culture *in vitro* as influenced by the concentration of NAA and BA. Using LSD test (p≤0.05).

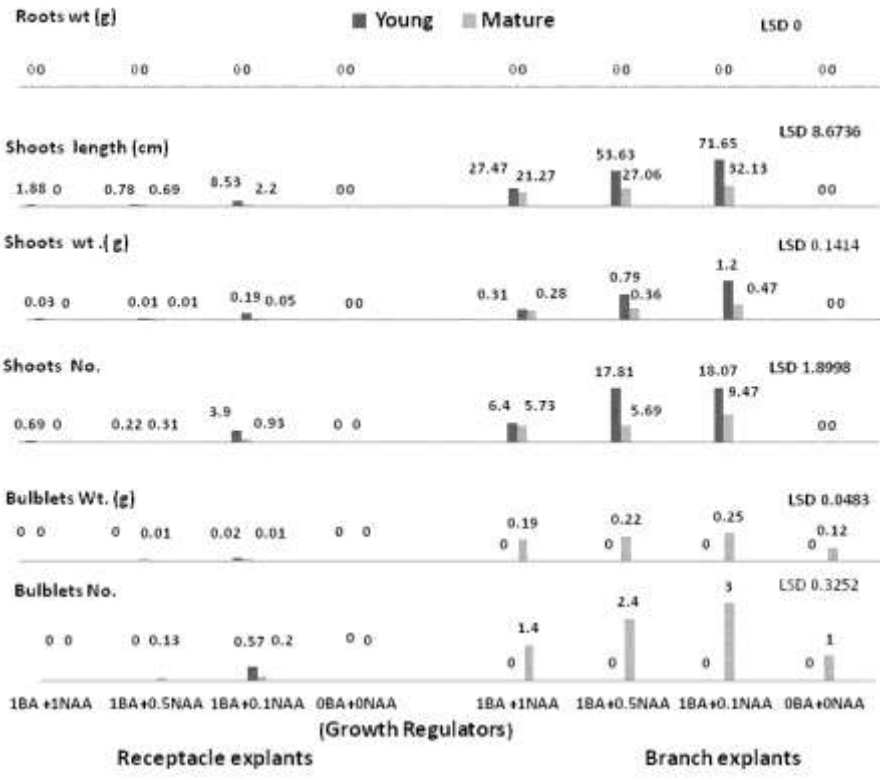

Figure 1D. Interaction effect of different concentration of plant growth regulators, type of explants and development stage of explants on the bulblets, shoots and roots regeneration of Asiatic lily in vitro. Using LDS test (p≤0.05).

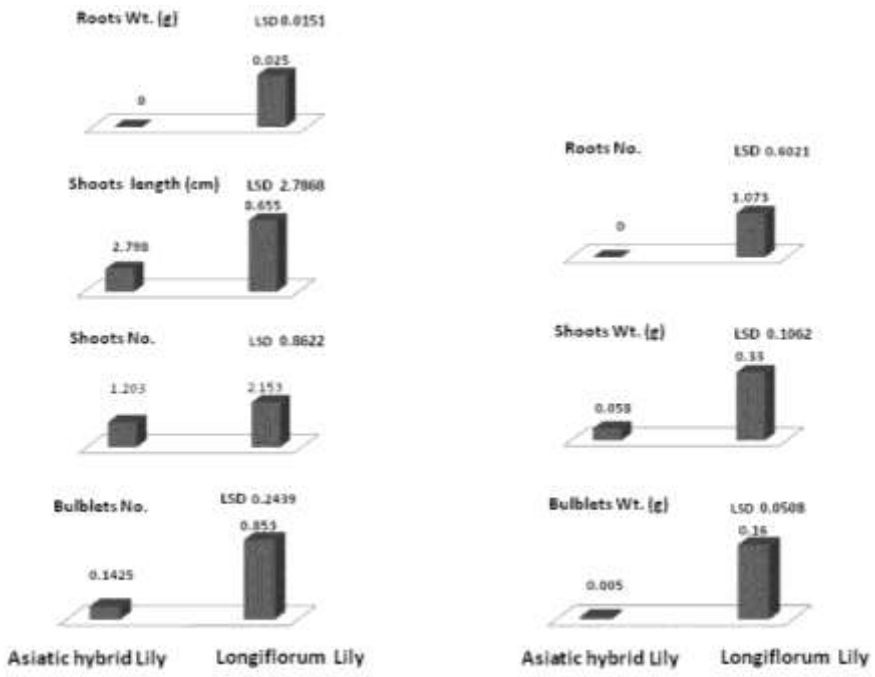

Figure 2A. Effect type of lily on the regeneration ability of explants after 6 weeks of culture *in vitro* using young receptacle explants using LSD test (p≤0.05).

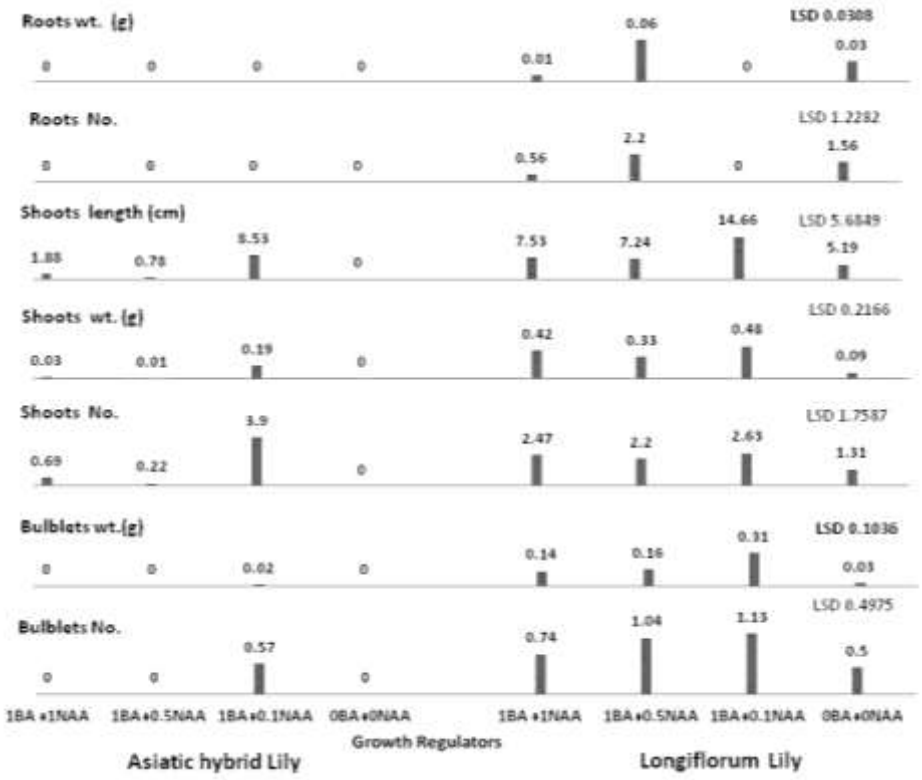

Figure 2B. Interaction effect of type of lily and different concentration of plant growth regulators on the bulblets, shoots and roots regeneration.

Plate 1. Floral reversion of Aslatic hybrid cv. Black out converting floral to vegetative organogenesis resulting in formation of a wide range of vegetative organs in young and mature receptacle explants after six weeks of *in vitro* culture using different concentration of naphthalene acetic acid (NAA) and benzyl aminopurine (BA). **A)** Great appearance of large bulblets on the boundary of receptacle-floral organs in young receptacle explants. **B)** Regeneration of multiple shoots on the boundary of receptacle-floral organs in young explants. **C)** Observation of buds and roots on the boundary of receptacle-flora organs in young explants. **D)** Formation of roots on young explants. **E)** Formation multiple roots in mature receptacle explants. **F)** The enlargements of ovary and shoot regeneration in mature receptacle explants.

Plate 2. Floral reversion of *Lilium longiflorum* cv. White heaven converting floral to vegetative organogenesis resulting information of a wide range of vegetative organs in young and mature receptacle explants after 6 weeks *in vitro* culture using different concentration of NAA and BA. **A)** Formation of large buds and shoots in young receptacle explants. **B)** Regeneration of shoots bulblets and roots in young explants. **C)** Observation of roots in the distal part of young explants. **D)** Formation of buds on the distal part of young receptacle explants. **E)** Appearance of abnormal shoots on the distal part of young explants. **F)** Development of shoots on the boundary of receptacle-floral organs of mature receptacle explants G-ovary enlargement and seeds formation were observed in mature receptacle explants.

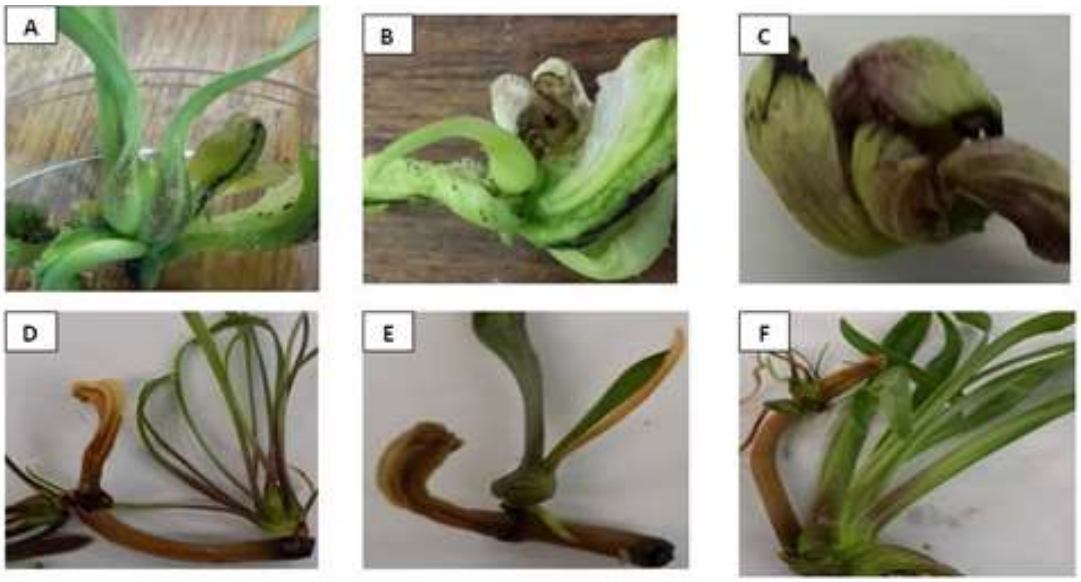

Plate 3. *In vitro* culture of Aslatic hybrid cv. Black out the bulblets and shoots rising from specific points. Flora organs-receptacle boundary and branching points of pedicle- peduncle in whole flora buds explants using different concentration of NAA and BA. **A)** Large bulblets appearance on the floral organs-receptacle boundary of young floral bud explants. **B)** Bulblet and shoots regeneration is associated with the floral organs degeneration. **C)** Floral reverse degeneration processes in young explants. **D)** Appearance of enlargement of whole ovary and the regeneration of bulblets and shoots on the both active points of mature explants. **E)** Large bulblets formation on the receptacle point of mature explants. **F)** Regeneration bulbelts, shoots and roots on the both active points of mature explants.

Plate 4. *In vitro* culture of Asiatic hybrid cv. Black out, after six weeks of culture in presence BA and NAA using young and mature branch explants of pedicel-peduncle, the result showed that the shoot and bulblets appeared only in branching points where pedicel join peduncle. **A)** Efficient regeneration of large multiple shoots on the young pedicle-peduncle branch explants. **B)** Development of shoots on the branching points in young explants. **C)** High performance of shoots regeneration of young explants. **D)** Bulblets and shoots regeneration on the branching points of mature pedicel-peduncle explants. **E)** Appearance of large bulblets on the point where the leave join the stem in mature explants. **F)** Development of large bulblet and shoots on the mature explants.

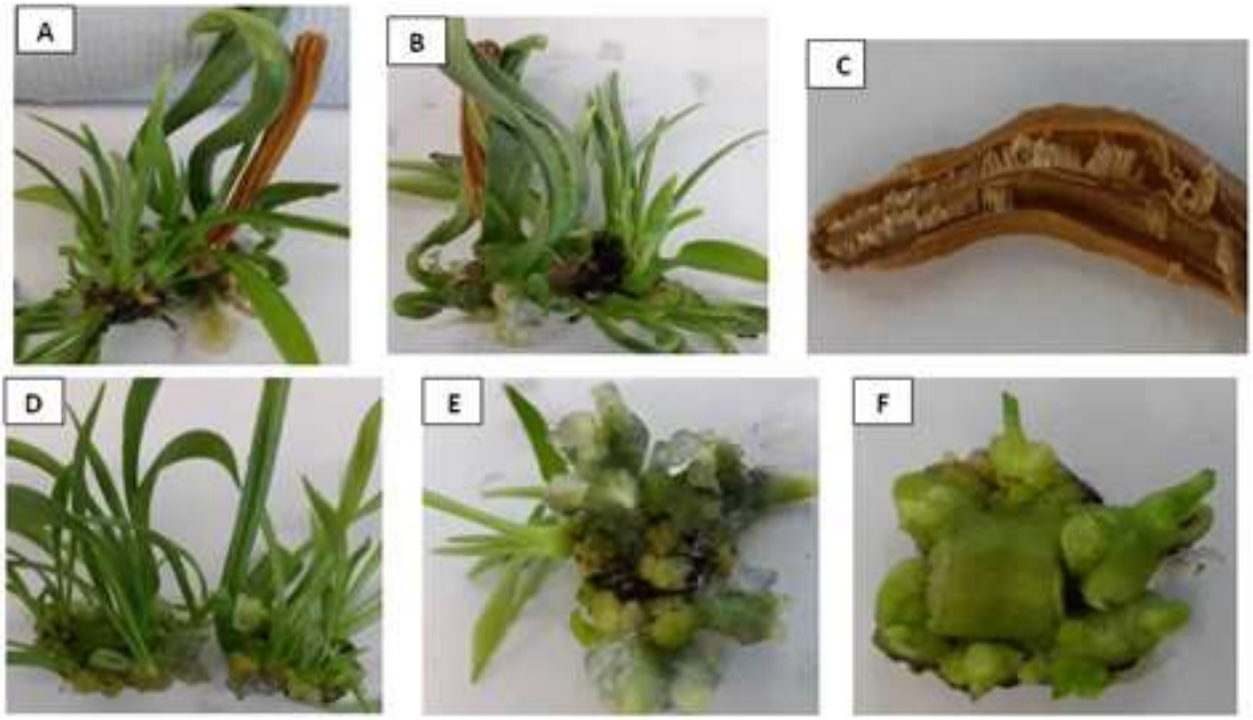

Plate 5. *In vitro* culture of *Lilium longiflorum* cv. White heaven, after 6weeks of culture using the mature whole floral buds explants and the mature receptacle explants with constant concentration of 1 mg/l BA combined with 0.1 mg/l NAA. A). Regeneration of shoots, bulblets and enlargements of ovary in mature whole floral buds explants. **B)** The vegetative organs regeneration in the boundary of receptacle-floral organs and distal part of receptacle in the mature whole flora bud explants. **C)** Seed formation inside ovary in mature whole flora buds explants. **D)** Shoots and bulblets regeneration in mature receptacle explants. E) Buds regeneration underneath adixal side which attached agar surface in mature receptacle explants. **F)** The mature receptacle explants were completely surrounded by the extensive numbers of buds and shoots on around cut surfaces.

The results of Figure 3A confirm that the whole floral bud explants had higher ability to regenerate bulblets, shoots and roots compared to receptacle explants. Figure 3B indicates that the regeneration ability of explants is highly dependent on all these experimental factors. However, the ovary enlargement was clearly observed in treatment of the mature receptacle of longiflorum lily.

DISCUSSION

Our previous study was concerned with the natural phenomenon of floral reversion in tiger lily *L. lancifolium* var. Flore Pleno, with high ability to produce inflorescence bulbils in nature (Asker, 2105). This present study investigates the artificial floral reversion processes in both lilies: Asiatic hybrid cv.”Black out" and *L. longiflorum* cv "White heaven”. They were enhanced by growth regulators under *in vitro* condition. Thus, many developmental changes were observed in response to this reversion when the regeneration of vegetative organs was associated with the degeneration of floral organs. Large bulblets and multiple shoots appeared in specific regions in floral organs, precisely in two attached points: the boundary region between the receptacle with other floral organs and the branching point where the peduncle joins the pedicel. This is in line with an histological study on *A. altissimum* (Poluboyarova et al., 2014) which reported that the morphogenic tissue in the area of fusion between stamens and sepals had the highest potency for direct shoot regeneration. This also agreed with the study of Ziv and Lilien- Kipnis (1997), which indicated that the pedicel - peduncle junction had high shoot regeneration in some geophytes.

That generated vegetative organs which appear only in specific points may be because the pedicel parts are different in their development process. Douglas and Riggs (2005) reported that the proximal portion and bulged distal region are different in their development in the model plant *(Arabidopsis thaliana*). Moreover, it was found that pedicle development process is under genetically control by several genes (Cho and Cosgrove, 2000; Kirik et al., 1998; Song and Clark, 2005; Ragni et al., 2008). The current results also showed that the direct

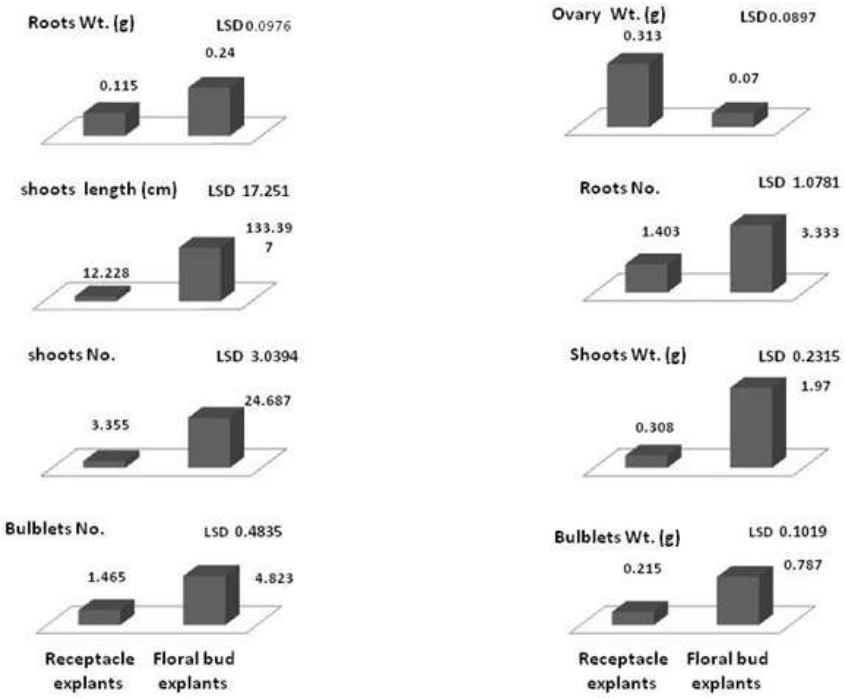

Figure 3A. Effect type of explants on the regeneration ability of explants after 6 weeks of of culture in vitro of both lilies using the concentration of 1mg/ 1BA combined with 0.1mg/1 NAA. Using LDS test (p≤0.05).

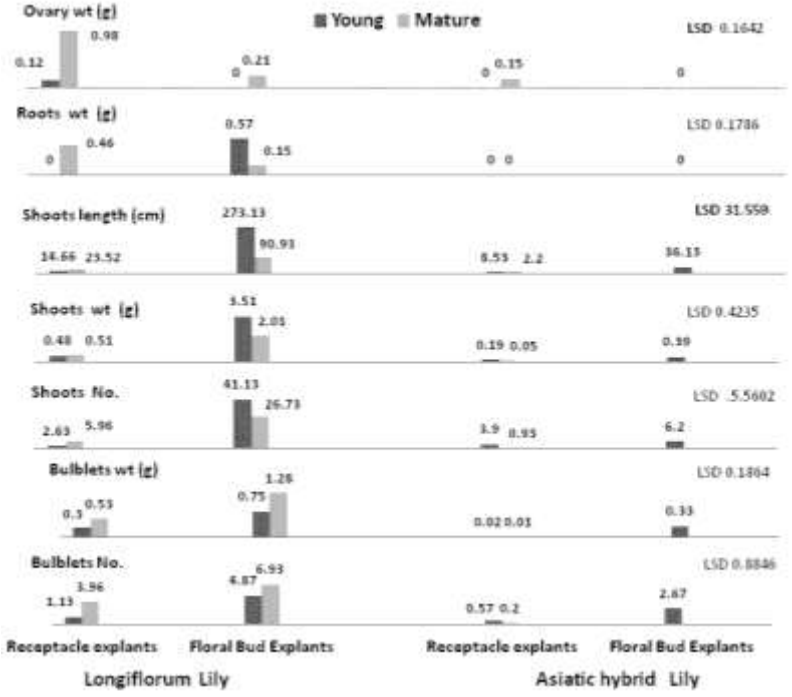

Figure 3B. Interaction effect of type of lily and the type and developmental stage of explants on the bulblets, shoot and roots regeneration after 6 weeks of culture in vitro using the concentration of 1mg/ 1BA combined with 0.1mg/1 NAA. Using LDS test (p≤0.05).

organogenesis which occurred in both lilies in response to floral reversion was highly dependent on type of lily, type of explants, developmental stage of explants in addition to the concentration of naphthalene acetic acid (NAA) and benzyl aminopurine (BA) *in vitro* culture.

L. longiflorum cv "White heaven" showed higher performance in regenerating bulblets and shoots and greater ability to enlarge ovary in response to floral reversion process compared to Asiatic hybrid cv."Black out". The inflorescence stalk branch explants and the whole floral bud explants produced more bulblets and shoots compared to the receptacle explants. Cytokinin (BA) combined with auxin (NAA) had great effect on the floral reversion process and subsequently on the performance of floral organs to regenerate bulblets, shoots and roots in *in vitro* culture. This result agrees with studies of Ziv and Lilien Kipnis (2000), Kumar et al. (2006), Werner and Schmulling (2009), Sakakibara (2006) and Bartrina et al. (2011) who demonstrated that these plant growth regulators play an essential role in plant morphogenesis *in vitro.*

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

I would like to express my appreciation to Professor M.P. Fuller for his invitation to visit the School of Biological Sciences, Plymouth University, Plymouth, Devon, PL4 8 AA, UK and to give me the opportunity to carry out my research work at his laboratory**.**

REFERENCES

Asker HM (2105). Inflorescence bulbils of tiger lily *in vivo* and bulbils culture *in vitro*. Afr. J. Biotech. 14(35):2616-2621

Bartrina IO, Elisabeth SB, Miroslav TS, Werner T, Thomas SL (2011). Cytokinin regulates the activity of reproductive meristems,flower organ size,ovule formation,and thus seed yield in Arabidopsis thaliana. Plant Cell 23:69-80.

Cho HT, Cosgrove DJ (2000). Altered expression of expansion modulates leaf growth and pedicel abscission in Arabidopsis thaliana. Proc. Nat. Acad. Sci. USA 97:9783-9788.

Douglas SJ, Riggs CD (2005). Pedicel development in Arabidopsis thaliana:contribution of vascular position and the role of the Brevipedicellus and Erecta genes. Dev. Biol. 284:451-463.

Kirik VK, KoIIe, Wohlfarth T, Miser AS, Baumlein H (1998). Ectopic expression of a novel MYB gene modifies the architecture of the Arabidopsis inflorescence. Plant J.13:729-742.

Kumar S, Kanwar JK, Sharma DR (2006). In Vitro propagation of Lilium. Adv. Hort. Sci. 20:181-188.

Liu L, Burger DW (1986). In vitro propagation of Easter lily from pedicels. Hort. Sci. 21:1437-1438.

Nhut DT, Bui VL, Jaime A, Silva TD, Teixeira DS, Aswath CR (2001). Thin cell layer culture system in Lilium: Regeneration and transformation perspectives. In vitro Cell Dev. Plant 37:516-523

Poluboyarova TV, Novikova TI, Vinogradova GY, Andronova EV (2014). Morpho-Histological analysis of direct shoot organogenesis induced in flower buds cultures of *Allium altissimum*. Am. J. Plant Sci. 5:2015-2022

Poluboyarova TV, Andronova EV, Novikova TI, Vinogradova GY (2011). Shoot Regeneration from floral tissues of *Allium altissimum* (Alliaceae). in Vitro culture. Plant Resour. 47:33-42.

Ragni L, Belles-boix E, Gunl M, Pautot V (2008). Interaction of KNAT6 and KNAT2 with Brevipedicellus and Pennywise in Arabidopsis inflorescence. Plant Cell 20:888-900.

Sakakibara H (2006). Cytokinins:Activity,biosynthesis,and translocation.Annu. Rev. Plant Biol. 57:431-449.

SAS (2012). Official methods of analysis system,Users guide. Statistical version 9.1th ed. SAS Inst.Inc.Cary,N.C.USA.

Song SK, Clark SE (2005). POL and related phosphatases are dosage-sensitive regulators of meristem and organ development in Arabidopsis, Dev. Biol. 285:272-284.

Supriyo S, Krishnasamy V, Krishnaveni S, Vijayalakshmi C, Rajamani K, Gnanam R. (2013). Evidence of floral revision during In Vitro Shoot regeneration from young inflorescence of the medicinal Geophyte Gloriosa superb L., Plant Tissue Cult. Biotechnol. 23:115-120.

Tooke F, Ordidge M, Chiurugwi T, Battery N (2005). Mechanisms and function of flower and inflorescence reversion. J. Exp. Bot. 56:2587-2599.

Werner T, Schmulling T (2009). Cytokinin action in plant development. Curr. Opin. Plant Biol. 12:527-538.

Ziv M, Lilien- Kipnis H (1997).The inflorescence stalk:Asource of highly regenerative explants for micrpropagation of Geophytes. Acta Hort (ISHS) 447:107-112.

Ziv M, Lilien- Kipnis H (2000). Bud re-generation from inflorescence explants for rapid propagation of Geophytes in vitro. Plant Cell Rep.19:845-850.