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# Effects of Polarity on Bulblet Regeneration on Stem Cuttings of Oriental Lilium Hybrid Cv. Casa Blanca

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**Abstract**

Oriental Lilium Hybrid cv. Casa Blanca is an important cut flower and garden plant. The study compared effects of *in vitro* bulblet induction on different oriented horizontally and vertically cultured cv. Casa Blanca stem explants with and without immersing them in agar solidified MS medium containing different concentrations of BAP. The results signified that orientation and immersing of explants into culture medium had significant effects on regeneration. Horizontally placed explants were advantageous over vertically placed upside up or upside down explants in terms of number of regenerated bulblets and their diameter. Maximum number of 6.84 ± 0.23 bulblets per "horizontally surface cultured stem explants" was noted on MS medium containing 0.20 mg/l BAP. These bulblets induced maximum mean bulblet diameter of 0.67 ± 0.02 cm Mean number of bulblets per explant was positively affected on horizontally cultured immersed explants. Contrarily, this parameters was negatively affected on vertically immersed upside up immersed and non immersed explants. However, vertically cultured upside down explants showed extreme inhibition with induction of bulblet initials only. The bulblets diameter was increased by culturing them on MS medium containing 4% sucrose for 8 weeks. Followed by their rooting on MS medium containing 0.5 mg/l IBA. They were acclimatized in greenhouse on peat moss prepared localy from leaves.

**Keywords:** Lilium hybrid, Casa Blanca, orienation, ornamental plant, mass propagation

## INTRODUCTION

Large attractive white flowered fragrant Oriental Lilium Hybrid cv. Casa Blanca is most widely sold Lilium species in Turkey after tulips. It is highly tolerant of summer heat and grow 3- 4 feet high. Its use as garden plant and as cut flower is well established [40]. It has hardy perennial bulbs that are easy to grow and are highly resistant to diseases.

Beattie and White, [13] has reported that it is reproduced vegetatively from bulb scales under *ex vitro* conditions. They report slow multiplication limits use of this method.

Tissue culture is employed as an alternative method to propagate bulbous plants [31, 43]. Although number of protocols and explants including bulb scales, flower pedicels, styles and filaments, pseudo-bulblets, bulb scales leaf segments [41, 42, 8, 28, 9, 21, 1, 46, 10, 23] are reported for propagation of these plants under *in vitro* conditions yet there is need to develop extended cost effective and fast regeneration tissue culture based multiplication protocols that must provide considerable genetic integrity [24, 27] during commercial production.

Plant regeneration is not as simple as understood and depends on the objectives of reproduction. Number of factors including genotype, composition of basal media, type of plant growth regulators, culture technique of explants, type of explants and their contact with culture medium affects success of micropropagation [19]. The study aimed to determine effects of explants orientation (horizontal placement {↔},vertical culture using stem explants in upside up position {↑} and upside down position {↓}) on *in vitro* multiplication of cv. Casa Blanca stem explants cultured under two different positions (immersion and non immersion) using [6]Benzyplaminopurine (BAP).

## MATERIALS and METHODS

Oriental Lilium Hybrid cv. Casa Blanca bulbs were purchased from Bauhaus Cepa AVM, Söğütözü, Ankara, Turkey. These bulblets contained 4 - 5 cm long profuse leaved stems containing 3 - 4 stem nodes. They were washed in detergent under tap water for 30 - 35 min after cutting and removing leaves. Thereafter, the stems were taken to laminar flow cabin that was disinfected with Dettol followed by rubbing with 96% ethanol. All experimental materials were also sterilized or autoclaved before use.

Each stem was divided into 0.90 -1.00 cm long segments. Care was taken to avoid portions close to stem nodes or carrying stem nodes. These were cultured/placed in two different positions (immersing 1-2 mm in the culture medium and non immersing) and three orientations horizontal placement {↔},vertical culture using stem explants in upside up position {↑} and upside down position {↓} (Fig. 1 a,b,c,d,e,f) on 0.65% (w/v) agar solidified MS medium containing 0.20, 0.40, 0.60, 0.80 and 1.00 mg/l BAP.

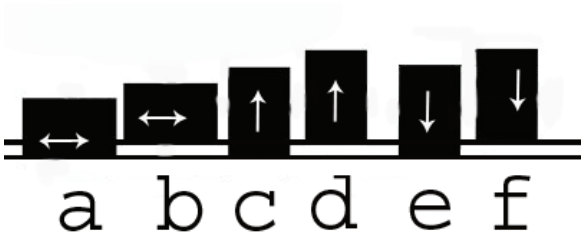


**Figure 1.** Placement of Oriental lilium hybrid cv. Casa Blanca stem explants for micropropagation (a) stem explants cultured horizontally by immersing 1 - 2 mm into the culture medium (b) stem explants not immersed into the culture medium and dropped on surface of the culture medium, (c) explants cultured vertically upside up or upside down by immersing 1 - 2 mm into culture medium, (d) explants not immersed in to the culture medium and dropped on surface of culture medium.

All explants in each treatment were incubated and cultured for eight weeks maintaining temperature of 24 ± 1 °C using 16 h light (42 µMol photons/$m^2$/s) photoperiod provided by day light lamps.

The pH of all culture media was adjusted to 5.7 ± 0.1 using 0.1 M KOH or 0.1 M HCl; which, were disinfected by autoclaving at 121°C and 104. 5 kPa pressure for 20 min.

**Rooting and hardening**

Rooting of *in vitro* regenerated bulblets was carried out on MS medium containing 0.5 mg/l IBA supplemented with 30 g/l sucrose and solidified with 0.65% (w/v) agar. The rooted plants were hardened on peat moss for 15 days and transferred to greenhouse for growth and development.

**Statistical analysis**

All experiments contained 60 explants divided in to 15 replications containing 5 explants per replication. The experimental developments were monitored periodically with final monitoring after eight weeks. The experimental data was measured for bulblet regeneration percentage, number of bulblets per explant and bulblet diameter. All experimental data was analyzed using one way ANOVA with the help of IBM SPSS 22 for windows. Standard error (±) of the means of each value was also calculated. To achieve normal distribution of curve all values obtained in percentage were arcsine transformed following methodology as described by Snedecor and Cochran [39].

## RESULTS

**Bulblet regeneration percentage**

Bulblet regeneration percentage is an important indicator of the success of a protocol. Although, no numerical differences were noted in regeneration percentage on horizontally cultured explants irrespective of immersion position and induced 100.00 ± 0.00% regeneration (Table 1). Statistically significant and reduced bulblet induction percentage was noted on 0.80 and 1.0 mg/l BAP on upside up immersed and 0.60, 0.80 and 1.00 mg/l BAP on upside up non immersed stems compared to other concentrations of BAP in respective columns. Whereas, each increasing concentration of BAP had inhibiting effect on regeneration percentage on upside down immersed and non immersed explants that induced bulblet initials induction in range of 39.89±0.6 - 92.44±0.20% and 22.38±0.13 - 87.50±0.33% respectively.

**Table 1.** Bulblet regeneration percentage of Lilium hybrid cv. Casa Blanca based on placement position, explant polarity and BAP concentrations

| BAP (mg/l) | Stems cultured horizontally by immersing 1-2 mm into culture medium | Stems cultured horizontally by placing explants on the surface of culture medium | Stems cultured vertically upside up by immersing 1-2 mm into culture medium | Stems cultured vertically upside up by placing explants on the surface of culture medium | Bulblet initials regeneration percentage on stems cultured vertically upside down by immersing 1-2 mm into culture medium | Bulblet initials regeneration percentage on stems cultured vertically upside down by placing explants on the surface of culture medium |
|---|---|---|---|---|---|---|
| 0.20 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00a | 100.00±0.00a | 92.44±0.20a | 87.50±0.33a |
| 0.40 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00a | 100.00±0.00a | 79.93±0.43b | 63.33±0.57b |
| 0.60 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00a | 83.33±0.09b | 69.33±0.79c | 46.71±0.81c |
| 0.80 | 100.00±0.00 | 100.00±0.00 | 83.33±0.42b | 66.67±0.05c | 53.14±0.17d | 39.61±0.37d |
| 1.00 | 100.00±0.00 | 100.00±0.00 | 66.67±0.65c | 25.67 ± 0.13d | 39.89±0.61e | 22.38±0.13e |

Values showed in a column by different small letters are significantly different using LSD test at 0.01 level of significance ± standard error

**Number of Bulblets per explant**

Number of bulblets per explant ranged 1.25 ± 0.24 to 4.10 ± 0.19, 1.80 ± 0.22 to 6.84 ± 0.23 (Fig 2 a, b), 1.56 ± 0.25 to 4.76 ± 0.23 and 1.14 ± 0.26 to 4.54 ± 0.31 (Fig 3a, b - Table 2) cultured horizontally, vertically upside up by immersing or non immersing stem explants respectively. Mean number of bulblets per explant was positively affected on horizontally cultured immersed explants. However, the vertically cultured upside down explants induced 0.13±0.01 to 3.72±0.21 and 0.25±0.02 - 3.15±0.29 bulblet initials per stem only that never matured or enlarged. Moreover bulblet initials induction on vertically immersed upside down culture of the explants using 0.20, 0.40 and 0.60 mg/l BAP in MS medium showed statistically non significant and negligible number of bulblet initials on 0.8 and 1.00 mg/l BAP irrespective of immersing. Maximum bulblet initials induction in case of each type of orientation and placement was noted on MS medium containing 1 mg/l BAP. Maximum number of 6.84 ± 0.23 bulblets per stem explant was induced by placing the explants on surface of the culture medium horizontally using 0.20 mg/l BAP that significantly differed in number of bulblet (4.10 ± 0.19) induced on next higher concentration of BAP on immersed stem explants cultured horizontally.

**Table 2.** Number of bulblets per explants of Lilium hybrid cv. Casa Blanca based on placement position, explant polarity and BAP concentrations

| BAP (mg/l) | Stems cultured horizontally by immersing 1-2 mm into culture medium | Stems cultured horizontally by placing explants on the surface of culture medium | Stems cultured vertically upside up by immersing 1-2 mm into culture medium | Stems cultured vertically upside up by placing explants on the surface of culture medium | Stems cultured vertically upside down by immersing 1-2 mm into culture medium | Stems cultured vertically upside down by placing explants on the surface of culture medium |
|---|---|---|---|---|---|---|
| 0.20 | 4.10±0.19aB | 6.84±0.23aA | 4.76±0.23aB | 4.54±0.31aB | 3.72±0.21aA | 3.15±0.29aB |
| 0.40 | 3.98±0.54aB | 5.79±0.48bA | 2.86±0.56bC | 2.24±0.34dC | 1.97±0.34bA | 1.42 ±0.71bB |
| 0.60 | 2.88±0.27bB | 4.24±0.74cA | 3.07±0.32bB | 2.46±0.66cB | 1.43±0.21cA | 0.93±0.22cB |
| 0.80 | 1.99±0.94cB | 2.74±0.04dA | 2.62±0.43bA | 1.66±0.89cB | 0.32±0.02dA | 0.53±0.05dA |
| 1.00 | 1.25±0.24eB | 1.80±0.22cA | 1.56±0.25cB | 1.14±0.26eB | 0.13±0.01dA | 0.25±0.02dA |

Values showed in a box horizontally by different capital letters are significantly different using t test at 0.01 level of significance
Values showed in a column by different small letters are significantly different using LSD test at 0.01 level of significance ± standard error

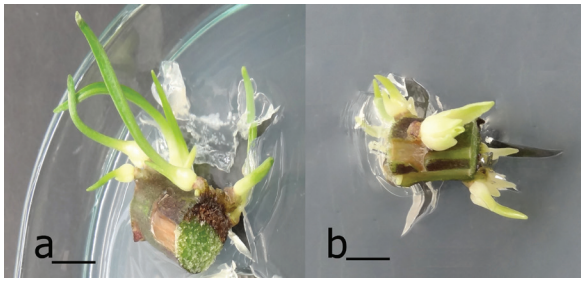


**Figure 2.** Bublblet regeneration on horizontally cultured explants (a) with and (b) without immersing into culture medium

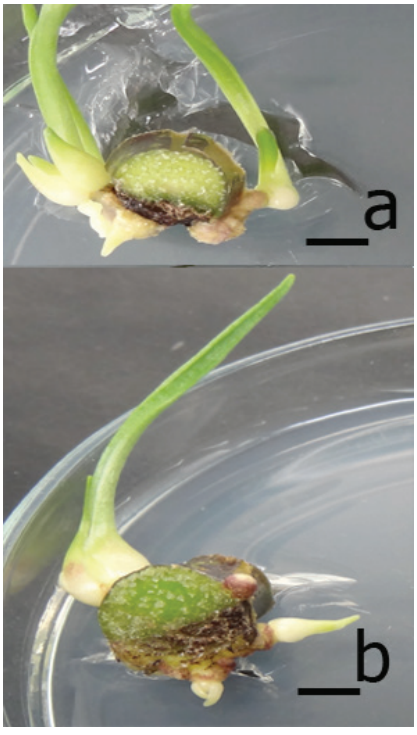


**Figure 3.** bublblet regeneration on vertically cultured explants (a) with and (b) without immersing into culture medium

**Bulblet diameter**

Bulb diameter had range of 0.21 ± 0.05 to 0.67 ± 0.02 cm, 0.31 ± 0.19 to 0.39 ± 0.05 cm, 0.38 ± 0.11 to 0.55 ± 0.16 cm and 0.14 ± 0.03 to 0.58 ± 0.19 cm on horizontally and vertically cultured upside up immersed or non immersed stems respectively. Bulblet initials were noted on stems cultured upside down, therefore, these were not measured for bulblet diameter. Although, bulblet diameters varied and had significant differences among them, largest bulblets on each type of placements and orientation were induced on 0.2 mg/l BAP treatment. The largest bulblets of 0.67±0.02 cm were noted on horizontally placed stems that were immersed into MS medium containing 0.2 mg/l BAP (Table 3).

Significant differences were noted in bulblet diameter when the explants were immersed versus no statistical difference noted on non immersed explants. Significant reduction in bulblet diameter were noted on immersed explants cultured vertically upside up using 0.80 and 1.0 mg/l BAP. Sharp decrease in induced bulblets diameter was noted with each increase in concentration of BAP in the culture medium on upside up vertically cultured non immersed explants. Minimum bulblet diameter was induced on each type of placement on MS medium containing 1 mg/l BAP. The bulblets induced average diameter of approximately 0.75 cm, when they were cultured on MS medium without isolating them from the mother explants imposing inhibition on neighbouring bulbs due to completion effect. However, average bulblet diameter increased to 1.25 cm when they were isolated and cultured individually on MS medium containing 3.0% (w/v) sucrose.

The growing bulblets were profusely rooted on 0.5 mg/l IBA without any problem in six weeks time. Thereafter, the bulblets were transferred to peat moss for acclimatization in 1 liter plastic pots. Peat moss was prepared locally from leaves. It had pH of 6.2 and EC of 0.12 ds $m^{-1}$ with high porosity and. The bulblets were secured with polythene packs in greenhouse under 16 h light photoperiod and temperature of 24˚ ± 2˚ C for acclimatization for 10 weeks (Fig 4).

**Table 3.** Average bulblet diameter of Lilium hybrid cv. Casa Blanca based on placement position, explant polarity and BAP concentrations

| BAP (mg/l) | Stems cultured horizontally by immersing 1 - 2 mm into culture medium | Stems cultured horizontally by placing explants on the surface of culture medium | Stems cultured vertically upside up by immersing 1-2 mm into culture medium | Stems cultured vertically upside up by placing explants on the surface of culture medium | Stems cultured vertically upside down by immersing 1-2 mm into culture medium | Stems cultured vertically upside down by placing explants on the surface of culture medium |
|---|---|---|---|---|---|---|
| 0.20 | 0.67±0.02aA | 0.39±0.05C | 0.55±0.16aB | 0.58±0.19aB | 0.00 | 0.00 |
| 0.40 | 0.55±0.12bA | 0.35± 0.06B | 0.49±0.14aA | 0.37±0.13bB | 0.00 | 0.00 |
| 0.60 | 0.39±0.16 cB | 0.33±0.03B | 0.47± 0.02aA | 0.30± 0.06bB | 0.00 | 0.00 |
| 0.80 | 0.39±0.05cA | 0.32±0.12B | 0.42±0.05bA | 0.23±0.01cB | 0.00 | 0.00 |
| 1.00 | 0.21±0.05dC | 0.31±0.19B | 0.38±0.11bA | 0.14±0.03dC | 0.00 | 0.00 |

Values showed in a box horizontally by different capital letters are significantly different using t test at 0.01 level of significance
Values showed in a column by different small letters are significantly different using LSD test at 0.01 level of significance ± standard error

**Figure 4.** Casa Blanca explants acclimatized under greenhouse conditions

## DISCUSSION

BAP has been recognised as an effective plant growth regulator for multiplication of many plant species [38, 20, 47, 29]. The experiment compared the effects of different orientations and placements of cv Casa Blanca stem explants on bulblet regeneration percentage. The experimental results showed that bulblet regeneration percentage was considerably affected by three different orientations and two placement positions (immersion and non immersion into the medium) that played an important role in inducing biological pattern of organogenesis [37]; with preferential expression of events in line Bloch, [14]. The results furher showed that BAP (Cytokinin) played an essential role in regulating cell divison, growth and development in cv. Casablanca. Placement of explants in different orientations affected mode of action of BAP that was modified inducing new orientations in stem cells or stem tissues leading to the differentiation of new vascular strands. Production of particular hormonal signals occured and disturbed based on orientation and environmental conditions (BAP concentration) provided during culture of explants. The experimental result further clearly indicate that Casa Blanca stem explants have remarkable regenerative abilities, however, they acted variably when they were cultured in three different orientations based on their placement. Maximum bulblets were induced on horizontally surface cultured stems followed by the stems that were cultured in vertical upside up position and least regeneration was noted on upside down cultured stems.

Generally, young shoots and roots are the main regions where auxins and cytokinins are produced regularly [5,4]). Each type of stem orientation received specific signals from each type of body in relation to orientation that activated different mechanisms in relation to structural structural pathways [3,6] regulating development and differentiation on stem explants. It was maximum when they were cultured horizontally followed by stems cultured vertically upside up that was inhibited or stopped, when they were cultured upside down. This affected their regeneration ability as well in agreement with Howell et al. [17], Rahayu et al. [32] and Werner et al. [44]. They found positive effects of cytkinins on shoot growth and development. However, vertically upside down cultured stem explants promoted multiplication of bulblet initials as apparantely BAP had difficulty in xylem movement and signalling for bulblet induction in explants [48,16,26,32] and did not promote cell divisions for induction of bulblets on the stem tissues [33]. The results are also in agreement with Sachs [37], who suggested that plant cells could behave variably during growth under different conditions of growth based on concentrations of BAP in the culture medium. It was clearly observed that explant orientation changed the behaviour of participating cells that indirectly controlled growth parameters depending on chemical interactions and distances in which diffusion was effective for each type of explant [15, 45, 25]. This diffusion or transport was variable and is related to an innate orientation of the tissues, that resulted in variable number of new bulblets or no bulblet induction that differed in diameter in agreement with Sachs [35] . Consequently, the number of bulblets per explant variably declined with increasing concentrations of BAP in relation to concentration and orientation of explants; even no bulblet induction was noted on vertically up side down cultured explants. It is also assumed that cytokinins stimulated vascular cambium activity in stem explants cultured horizontally or vertically upside up by promoting vascular regeneration with increased number of phloem and xylem strands around the explant wound [7, 12, 2].

Immersing of the explants either in case horizontal or vertical placements were inhibitory. Similarly, immersing of

the explants vertically upside down were inhibitory. Placing explants on the surface of the culture medium was always advantageous with induction of more bulblets per explants compared to the situations when the explants were immersed in to the culture medium. The results clearly demonstrate that the stem explants induced differentiation in vascular development and induced new meristems based on their position along new axes affected by their positions and orientations, that would not have changed in undisturbed tissues in agreement with Sachs [37]. The variable bulblet induction behavior, number of bulblets per explant and bulblet diameter specific to the orientation or placement and concentration of BAP molecules in the culture medium is in agreement with Jacobs, [18]; Sachs, [34, 36, 37]; who had such observations in shoot induction .

Concentration of BAP in the culture medium controlled differentiation of cells in relation to explant orientation or placement and concentration of BAP that patterned the distribution of the signals, which in turn determined further differentiation of positive or negative feedbacks.

It was not difficult to increase bulblet diameter on MS medium containing 4% sucrose. Sucrose has been also been reported to increase bulb diameter in other bulbous plant species like Fritillaria persica [22].

Previous reports suggest use of IBA to root in vitro regenerated bulbs in agreement with Ozel and Khawar [30] and Sevimay et al. [38].

The plants were acclimatized using peat moss under greenhouse conditions. Peat moss is an important substrate with high porosity that helps to retain proper soil moisture, effective growth of roots, and growth of plants under in vitro conditions in agreement with Barpete et al. [11].

## CONCLUSION

In conclusion, it was found that bulblets of Cv Casa Blanca can be produced in quantity in a relatively short time using horizontally and up side up vertically placed stems on surface of the culture medium and grown to make flower bulbs.

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