High performant eggplant *in vitro* regeneration and organogenesis

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ABSTRACT Eggplant is a notable vegetable crop grown in a variety of tropical and temperate regions. *In vitro* regeneration of eggplant was established to determine an efficient phytohormone concentration for development of organogenesis from cotyledon and leaf explants. Ten-day old cotyledon and 25-day old leaf explants were cultured on MS medium amended with Gamborg's B_5 vitamins and 2% sucrose in 10 different concentrations and combinations of NAA, BAP, TDZ, 2,4-D and IAA phytohormons. Cultured explants were incubated at 25 ± 2 ºC for 3 weeks under 16:8 h photoperiod. Well-grown regenerated shoots were transferred to fresh growth medium after 3 weeks for shoot elongation. All cotyledon and leaf explants produced various callus masses but only white and friable ones were able to regenerate into vigorous shoots. Shoot regeneration medium (SRM_{10}) containing MS + TDZ 2 mg/L + BAP 0.5 mg/L + NAA 0.5 mg/L showed high potential in organogenesis of both explant types. Shoot regeneration and organogenesis from cotyledon explants were much more advantageous than leaf explants. The average of cotyledon and leaf explants producing shoot were 4.3 ± 0.33 and 1.6 ± 0.67 out of 5 in each plate, respectively. The percentages of regenerated shoots in cotyledon and leaf explants cultured on SRM_{10} were 86.6 ± 6.67 and 33.3 ± 13.3%, respectively. Current work can shed a light on production of transgenic eggplants and can be followed by normal mature plants regeneration.

Keywords:
- *Solanum melongena*
- tissue culture
- phytohormones
- cotyledon explant
- leaf explant
- shoot regeneration

Introduction Eggplant (*Solanum melongena* L.) or *aubergine* as it is called in France, is a vegetable long prized for its beauty as well as its unique taste and food value. Significantly, more than 4,000,000 acres of farmlands were devoted to eggplant cultivation in the world[4] and farm gate was value of $37 million.[23] It has been used as a food full of vitamins and also as a traditional medicine.[11] Eggplant has been cultivated in Asia for over 1500 years and its germplasm resources and collections have been well documented, evaluated and conserved throughout the world.[19] Biotechnology applications in eggplants were launched by *in vitro* tissue culture and regeneration development.[3,8,18] Eggplant could easily be regenerated through *in vitro* organogenesis[13,20] and somatic embryogenesis[10,20] from cultured explants provided from stem, hypocotyl, leaf, cotyledon and root, also from cell suspension, anthers[9], isolated microspores [15], and protoplasts.[22] Explant regeneration in eggplant has been performed using media supplemented with benzyladenine[7], zeatin[7], kinetin[7], and thiadiazuron (TDZ)[13] for organogenesis, and α-napthaleneacetic acid[7,20] for somatic embryogenesis. Efficient regeneration of other species of *Solanum* was obtained from cotyledon explants culture.[6] Using of TDZ enhances shoot organogenesis; the leaves and cotyledons development mostly influenced by TDZ.[13] Advances in laboratory methodologies along with eggplant high potential for tissue culture, particularly in regeneration of cultured leaf, cotyledon and hypocotyl segments have allowed development and implementation of various powerful biotechnological techniques implementation in genetic resources management and improvement. The objective of the present research was performing a high efficient *in vitro* shoot organogenesis of eggplant using two types of eggplant explant and determination of a suitable medium in which hormone concentrations can help to explants to be regenerated.

Materials and Methods

Eggplant seeds provided from Forest Research Institute of Malaysia (FRIM) superficially sterilized dipping in 70% EtOH for 1 min, followed by 25% commercial bleach along with 5.25% of sodium chloride for 20 min and washed three times with sterilized distilled water and cultured on MS medium[16] supplemented with basal salts, B_5 vitamin[5] and 2% of sucrose. Seeds were incubated at 25 °C in cultural tubes containing 30 ml of hormone-free MS medium for three days in darkness, followed by a photoperiod of 16:8 hours (L:D) to acquire cotyledon and leaf explants. Cotyledon and leaf tissues obtained respectively from 10 and 25-day old seedlings were used as explants. Cotyledon explants were prepared in 5 × 5 mm and leaf explant in 10 × 5 mm in size using sterile scalpel on sterile filter paper from the center of cotyledon tissue. Cotyledon and leaf segments were cultured on culture media in Petri dish (90 × 25 mm) (Table 1) and each plate was consisted of five explants in three replications (Figure 1). Plates were kept at 25 ºC under a photoperiod of 16:8 hours for three weeks. The regenerated shoots were excised from explants and sub-cultured on fresh medium for additional shoot development. The type, volume and color of callus were exactly assessed by direct observation with stereomicroscope. The number of shoots developed from the callus was counted. Standard errors were calculated and the means compared using SPSS software. All graphs were drown using Graphpad Prism 6 software.

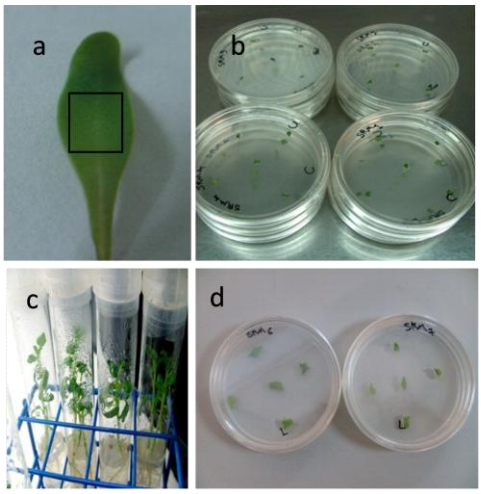

Figure 1. Cotyledon and leaf explants preparation and culture in shoot regeneration media. a) centre of cotyledon tissue for explant preparation b) explants-eggplant seedling d) Leaf explants culture in MS media

Results

Effect of phytohormones on explants regeneration

The results of tissue culture establishment showed high performance of shoot regeneration on SRM_{10} used for the entire of experiment, afterward. The volume of callus initiation in SRM_3, SRM_4, SRM_8 and SRM_{10} were higher than cotyledon-cultured in other MS media (Table 2). Callus volume was higher on SRM_4 and SRM_{10} than others indicating that cotyledon was more flexible tissue than leaf for *in vitro* culture and regeneration. Most of the produced callus type on SRM_{10} medium was friable and soft which was most efficient callus type for shoot organogenesis, also callus color in SRM_{10} was white and creamy and these type of callus efficiently produced shoot. The percentage of cotyledon explants produced shoot was higher than leaf explants in shoot organogenesis and it was because of more competent callus production potential for shoots generation.

In cotyledon explants, no shoot initiation and competent callus formation was able to produce shoot on SRM_1, SRM_3 and SRM_7 media but SRM_{10} showed the highest percentage of explants, producing shoots (Figure 2). In leaf explants, the number of explants produced shoot was high on SRM_4 and there was no shoot formation on SRM_1 and SRM_7 (Table 2).

The higher percentage of cotyledon explants producing shoots was on SRM_{10}. Leaf explants showed high potential in shoot developing on SRM_4. Overall, the average number and percentage of cotyledon explants produced shoot were higher than leaf explants and potential of cotyledon explants were more than leaf explants for *in vitro* shoot organogenesis.

Shoot organogenesis

Small buds were generated at the cut edge of eggplant cotyledonary explants after three weeks followed by regeneration of shoots on SRM_{10} medium (Figure 2). It was estimated

Table 1. Plant growth regulators combination and concentrations in MS

MS Medium	Plant Growth Regulators (mg/L)				
	NAA	2,4-D	IAA	TDZ	BAP
SRM_0 (Control)	-	-	-	-	-
SRM_1	-	-	-	0.5	-
SRM_2	-	-	-	1	-
SRM_3	-	-	-	-	0.5
SRM_4	-	-	-	-	1
SRM_5 [17]	-	-	0.5	0.1	2.5
SRM_6	0.5	-	-	-	2.5
SRM_7 [20]	-	0.5	-	-	2.5
SRM_8 [1]	-	0.3	-	-	-
SRM_9	0.3	-	-	-	-
SRM_{10}	0.5	-	-	2	0.5

Table 2. Effect of different plant hormone concentrations in MS media on shoot organogenesis of eggplant cotyledon and leaf explants

explant	MS medium concentration	number of explants produced shoot	explants produced shoot (%)	callus color	callus volume	callus type
cotyledon	SRM_0 (Control)	0.3 ± 0.33*	6.6 ± 6.67	LB/Y	++	SC/CC
	SRM_1	0 ± 0.00	0 ± 0.00	B	+	CC
	SRM_2	0.6 ± 0.33	13.3 ± 6.67	LB/W	++	CC/FC
	SRM_3	0 ± 0.00	0 ± 0.00	B/Y	+++	CC
	SRM_4	1 ± 0.00	20 ± 0.00	W/Y	+++	CC/FC
	SRM_5	1 ± 0.58	20 ± 11.55	LB/W	++	SC/CC
	SRM_6	0.6 ± 0.67	13.3 ± 13.33	LB/W	+	CC
	SRM_7	0 ± 0.00	0 ± 0.00	B	+	CC/FC
	SRM_8	1 ± 0.58	20 ± 11.55	W/Y	+++	FC/SC
	SRM_9	1 ± 0.58	20 ± 11.55	LB	++	CC/FC
	SRM_{10}	4.3 ± 0.33	86.6 ± 6.67	W/C	+++	FC/SC
leaf	SRM_0 (Control)	0 ± 0.00*	0 ± 0.00	B/LB	+	CC
	SRM_1	0 ± 0.00	0 ± 0.00	B	+	CC
	SRM_2	0.3 ± 0.33	6.6 ± 6.67	B/W/G	++	CC
	SRM_3	0.6 ± 0.67	13.3 ± 13.3	B/W	++	CC/FC
	SRM_4	2 ± 1.00	33.3 ± 20.00	W/Y	+++	FC/CC
	SRM_5	0.6 ± 0.67	13.3 ± 13.3	LB/W	++	CC/SC
	SRM_6	0 ± 0.00	0 ± 0.00	B/G	+	CC
	SRM_7	0 ± 0.00	0 ± 0.00	B	+	CC
	SRM_8	0.3 ± 0.33	6.6 ± 6.67	B/W	+	FC/CC
	SRM_9	0.3 ± 0.33	6.6 ± 6.67	LB/W	++	CC/SC
	SRM_{10}	1.6 ± 0.67	33.3 ± 13.3	W/C/LB	+++	FC/SC

SRM: Shoot Regeneration Medium. Callus Color: B = Brown, LB = Light Brown, C = Creamy, G = Greenish, LG = Light Green, DG = Dark Green, W = White, Y = Yellow, LY = Light Yellow. - = No Callus and no shoot. Callus Degree: + = Slight Callus, ++ = Moderate Callus, +++ = Massive Callus. 0 & - : tissue necrosis. Callus Quality: FC = Friable Callus, SC = Soft Callus, CC = Compact Callus. The mean ± standard error of three replicated with five explants per treatment in each experiment. data are given as mean ± standard error

that the best stage of soil acclimatization of transformants would be after the shoot elongation and root formation (Figure 2).

Root formation and soil acclimatization

After three weeks some eggplant plantlets were rooted and some others were needed to be sub-cultured on fresh rooting medium for additional root development. Rooted shoots were transferred into the plastic pots filled with sterilized soil, sand and vermiculite (1:1:1, v/v/v) for soil acclimatization and plant development.

Discussion

In vitro regeneration and shoot organogenesis of eggplant carried out in current research, shoot organogenesis was only achieved in white and friable callus consisting of large and succulent cells which could actually produce shoots.[12,21] Compact, greenish and brown callus was not able to produce shoot; however, they were developed during culture days. Obviously, shoot regeneration was initiated from white and friable callus masses in this research shown in Figure 2. Addition of high concentrations of sucrose to MS medium caused early necrosis at the cut edges of explants. It might be a result of phenolics accumulation at the cultural condition which caused necrosis traces on the edges of explants.[24] Rotino and Gleddie (1990) used 1% of sucrose in B_5 medium preparation for eggplant transformation and regeneration. In contrast, Billings *et al*., (1997) used 2% sucrose in MS medium preparation in order to culture the eggplant leaf and stem segments and in this research only 2% of sucrose was used in medium preparation for the entire of experiment and no necrosis symptoms on the edge of explants were observed. However, the standard level of sucrose in MS medium is considered 3%.[16] In cotyledon explant culture, the central section of cotyledon tissue was used as explants in present work for its suitable cells and using different concentration of plant hormones effected on the number of explants producing shoot from white and friable callus. We found

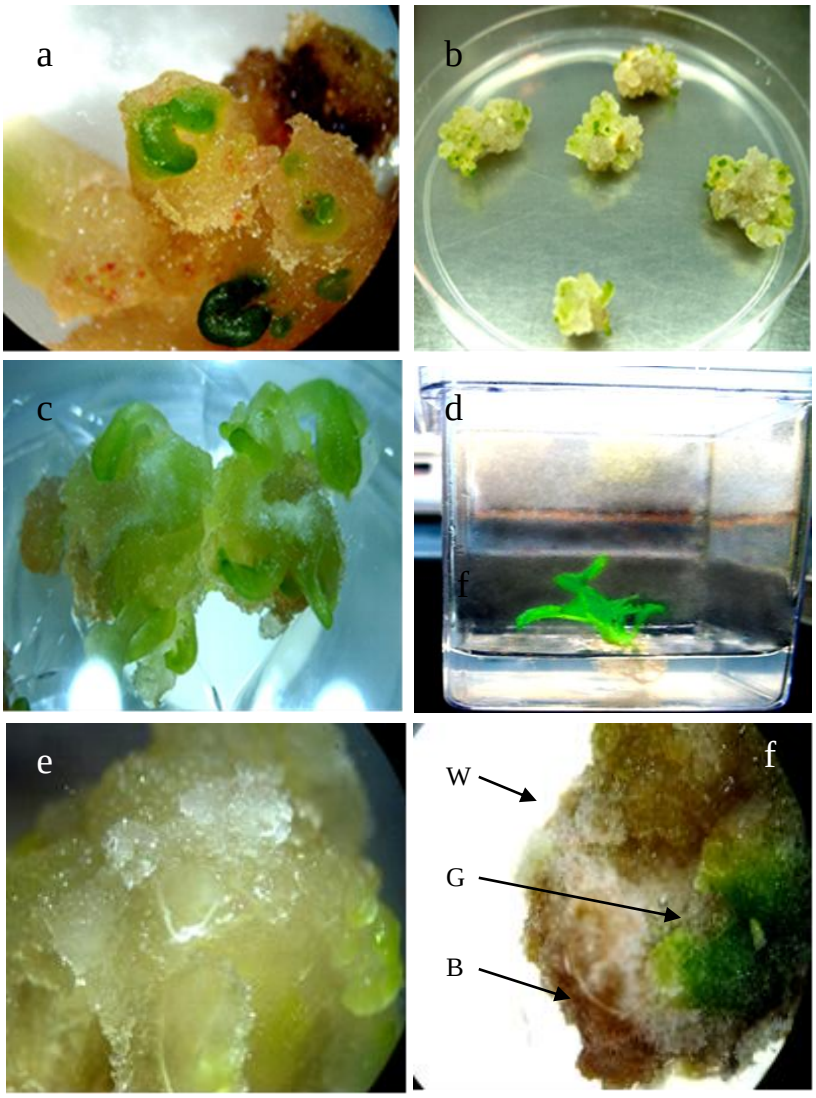

Figure 2. Shoot regeneration and development of eggplant in SRM_{10} media. a) small buds formed from friable and white callus. b and c) small buds developing and shoot organogenesis. d) root formation of regenerated shoot. Arrows show regenerated shoot e) white and friable callus f) Brown (B), greenish (G) and white (W) callus formation in cultured explants

that TDZ was more efficient than BAP in shoot regeneration of eggplant, also NAA was effective than the other type of auxins in this experiment particularly in combination with BAP. Organogenesis in eggplant has been induced on media containing NAA or TDZ.[13] Although, there are several research reported a negative effect of TDZ on shoot elongation in eggplant[14]; hence SRM_{10} was the best option for regeneration and developing of initiated shoots from white and friable callus masses in about all genotypes of eggplant particularly commercial traits. In conclusion, recognition of highly regenerative explant type, effective hormone type and concentration in medium for shoot regeneration of eggplant can be very helpful to developing transgenic eggplant be successful in production of transgenic eggplant in future as *in vitro* tissue culture and shoot organogenesis is a prerequisite; however environmental factors and plant genotype[20] were not assessed in this research.

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References

1. Alicchio R, Del Grosso E, Boschieri E (1982) Tissue cultures and plant regeneration from different explants in six cultivars of *Solanum melongena*. Experientia 38: 449 – 450.
2. Billings S, Jelenkovic G, Chin C-K, Eberhadt J (1997) The effect of growth regulation and antibiotics on eggplant transformation. Journal of American Society Horticultural Science 122: 158–162.
3. Fari M, Nagy I, Csanyi M, Mitkyo J, Andrasfalvy A (1995) *Agrobacterium*-mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (*Solanum melongena* L. cv. 'Kecskenmefi lila'). Plant Cell Report 15: 82-86.
4. FAOSTAT (2012) Food and Agriculture Organization of the United Nations (FAO) Statistical Databases. Available on-line as <http://www.fao.org.> on 12 May 2012.
5. Gamborg OL, Miller RA, Ojima K (1968) Nutrient Requirements of Suspension Cultures of Soybean Root Cell. Experimental Cell Research 50: 151-158.
6. Gisbert C, Prohense J, Nuez F (2006) Efficient regeneration in two potential new crops for subtropical claimates, the scarlet (*Solanum aethiopicum*) and gboma (*S. macrocarpon*) eggplant. New Zealand Journal of Crop and Horticultural Science 34: 55-62.

7. Gleddie S, Keller W, Setterfield G (1983) Somatic embryogenesis and plant regeneration from leaf explants and cell suspensions of *Solanum melongena* (eggplant). Canadian Journal of Botany 61: 656-666.
8. Iannacone R, Fiore MC, Macchi A, Grieco PD, Arpaia S, Perrone D, Mennella G, Sunseri F, Cellini F, Rotino GL (1995) Genetic engineering of eggplant (*Solanum melongena* L.) Acta Horticulture 392: 227-233.
9. Isouard G, Raquin C, Demarly Y (1979) Obtention de plantes haploïdes et diploïdes par culture *in vitro* d'anthères d'aubergine (*Solanum melongena* L.). Acad Science Paris 288: 987–989.
10. Kalloo G (1993) Eggplant (*Solanum melongena*). Genetic improvement of vegetable crops. Pergamon Oxford Pages: 587–604. Available from: <https://books.google.com.tr/books?id=oh1S2zLjAGsC&dq=+Genetic+improvement+of+vegetable+crops.+Pergamon,+Oxford.&lr=&hl=tr&source=gbs_navlinks_s>
11. Khan R (1979) *Solanum melongena* and its ancestral forms. In: Hawkes J.C, Lester R.N, Skelding A.D (eds). The biology and taxonomy of *Solanaceae*. Linnean Society Academic Press London, Pages: 629–636.
12. Konwar BK, Coutts RHR (1990) Rapid regeneration of sugarbeet (*Beta vulgaris* L.) plants from *in vitro* cultures. Current Plant Biotechnology Science 114-118.
13. Magioli C, Rocha APM, De oliveira DE, Mansu E (1998) Efficient shoot organogenesis of eggplant (*Solanum melongena* L.) induced by thidiazuron. Plant Cell Report 17: 661–663.
14. Marcotrigiano M, McGlew SP, Hackett G, Chawla B (1996) Shoot regeneration from tissue-cultured leaves of the American cranberry (*Vaccinium macrocarpon*). Plant Cell Tissue and Organ Culture 44: 195–199.
15. Miyoshi K (1996) Callus induction and plantlet formation through culture of isolated microspores of eggplant (*Solanum melongena* L.). Plant Cell Report 15: 391–395.
16. Murashige T, Skoog FA (1962) Revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology 15: 473-49.
17. Pratap D, Kumar S, Raj KS, Sharma KA (2011) *Agrobacterium*-mediated transformation of eggplant (*Solanum melongena* L.) using cotyledon explants and coat protein gene of Cucumber mosaic virus. Indian Journal of Biotechnology 10: 19-24.
18. Rotino GL, Gleddie S (1990) Transformation of eggplant (*Solanum melongena* L.) using a binary *Agrobacterium tumefasciens* vector. Plant Cell Report 9: 26-29.
19. Sarathbabu B, Varaprasad K S, Charaabarty S K, Sivaraj N (1999) Status of resistance germplasm in eggplant (*Solanum melongena* L.) and wild *Solanum* species. Indian Journal of Plant genetic Resource 12: 56-64.
20. Sharma P, Rajam MV (1995) Genotype, explant and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). Journal of Experimental Botany 46: 135–141.
21. Shimamoto Y, Hayakawa H, Abe J, Nakashima H, Mikami T (1993) Callus induction and plant regeneration of beta germplasm. Journal of Sugar Beet Resource 30: 317-319.
22. Sihachakr D, Daunay M C, Serraf I, Chaput M H, Mussio I, Haicour R, Rossignol L, Ducreux G (1994) Somatic hybridization of eggplant (*Solanum melongena* L.) with its close and wild relatives. Springer-Verlag, Berlin, Heidelberg. 1: 255–278.
23. USDA (2003) Vegetables 2002 summary, Agricultural Statistics Board, National Agricultural Statistics Service. Available on-line as <http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Vegetables/>
24. Yildiz M, Onde S, Ozgen M (2007) Sucrose effects on phenolic concentration and plant regeneration from sugar beet leaf and petiole explants. Journal of Sugar Beet Resource 44: 1-15.

باززایی و اندام‌زایی با عملکرد بالای بادمجان در محیط کشت مصنوعی

مجله بوم‌شناسی گیاهان زراعی
جلد ۱۱، شماره ۲، صفحات ۲۹ -۲۴
(تابستان ۱۳۹۴)

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چکیده بادمجان یک گیاه جالیزی مهم که در مناطق مختلف گرمسیری و معتدل رشد می‌کند. باززایی بادمجان در محیط مصنوعی جهت تعیین کردن غلظت مناسب و کارآمد هورمون‌های گیاهی به منظور اندام‌زایی از بافت کوتیلدون و برگ انجام شد. بافت کوتیلدون ۱۰ روزه و بافت برگ ۲۵ روزه در محیط کشت MS حاوی ویتامین‌های B_5 و ۲٪ ساکارز و در ۱۰ غلظت مختلف از هورمون‌های NAA, BAP, TDZ, 2,4-D و IAA کشت گردیدند. بافت‌های کشت شده در دمای ۲ ± ۲۵ درجه سلسیوس تحت ۱۶:۸ ساعت روشنایی و تاریکی قرار گرفتند. اندام‌های خوب باززایی شده (ساقه) بعد از سه هفته به محیط کشت جدید جهت افزایش طول ساقه منتقل شدند. تمام بافت‌های کوتیلدون و برگ توده‌های کالوس یا بافت‌های متمایز نشده مختلفی تولید کردند ولی تنها کالوس‌های سفید و ترد قادر به تولید جوانه‌های قوی بودند. بالاترین بازدهی محیط کشت مربوط به محیط کشت جوانه SRM_{10} که محیط کشت MS حاوی ۲ میلی‌گرم در لیتر TDZ، ۰/۵ میلی‌گرم در لیتر BAP و ۰/۵ میلی‌گرم در لیتر هورمون NAA بود که بیشترین مقدار بازدهی و تولید را در هر دو نوع بافت نشان داد. ساقه-زایی و اندام‌زایی از بافت کوتیلدون خیلی بیشتر از بافت برگ بوده و به طور متوسط از هر پنج بافت کوتیلدون و برگ در هر ظرف پتری به ترتیب ۰/۳۳ ± ۴/۳ و ۰/۶۷ ± ۱/۶ بافت اندام‌زایی ایجاد کردند. درصد باززایی بافت کوتیلدون و برگ در محیط کشت SRM_{10} به ترتیب ۶/۶۷ ± ۸۶/۶ و ۱۳/۳ ± ۳۳/۳ بود. این تحقیق می‌تواند راه حلی جهت تولید بادمجان تراریخته ارایه داده و باززایی و تولید گیاهان بالغ را در محیط مصنوعی ایجاد نماید.

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- **بافت برگ**
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