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Effects of Different Plant Hormones on Callus Induction and Root Formation of Eggplant (*Solanum melongena* L.)

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Abstract:

A study was undertaken in the tissue culture laboratory of the Genetic Engineering Department, Directorate of Agricultural Research, Ministry of Science and Technology, to explore the effects of different plant growth regulator (PGR) combinations on the induction of callus, root formation, and acclimatization in *Solanum melongena* L. A completely randomized design was employed in a factorial experiment with five replications. The results revealed that, increasing concentrations of gibberellic acid increased germination percentage and seedling length, while germination time decreased. Additionally, results showed superiority in callus induction with the combination of 2 mg l⁻¹ kinetin (Kn.) + 2 mg l⁻¹ Benzyl adenine (BA) + 2 mg l⁻¹ Indole acetic acid (IAA). Root formation varied from 41% to 83% and 1mg l⁻¹ Indole butyric acid (IBA) recorded best significant values. The regenerated plantlets were hardened and acclimatized, and achieving a rate of 90% survivability.

Key words: Auxins, Cytokinins, Callogenesis, Eggplant, Root formation

استئثار الكالس وتكوين الجذور باستخدام توليفات هرمونية مختلفة من نبات البازنجان (*Solanum melongena* L.)

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الخلاصة

اجريت هذه الدراسة في مختبر الزراعة النسيجية العائد الى قسم الهندسة الوراثية، دائرة البحوث الزراعية، وزارة العلوم والتكنولوجيا، للكشف عن تأثيرات توليفات مختلفة من منظمات النمو النبات على حد الكالس وتكوين الجذور واقلمة نبات البازنجان. استخدم التصميم العشوائي الكامل في تجربة عاملية وبخمسة مكررات. اظهرت النتائج ان زيادة تراكيز حامض الجيرلين زادت من نسبة الانبات وطول البادرات بينما انخفض زمن الانبات. بالإضافة الى ذلك اظهرت النتائج ايضا لوحظ تفوقا في حد للكالس في التوليفة المركبة من 2 ملغم لتر⁻¹ كابينتين + 2 ملغم لتر⁻¹ بنزيل ادينين + 2 ملغم لتر⁻¹ اندول حامض الخليك. تراوحت تكوين الجذور من

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الى 83% وسجل التركيز 1ملغم لتر⁻¹ من اندول حامض البيوتريك افضل القيم معنويا. تم تقسيمة النباتات الناتجة واقلمتها، وحققت معدل بقاء 90%.

1. Introduction

Eggplant (*Solanum melongena* L.) ranks as a significant vegetable globally, following potato and tomato and belongs to the Solanaceae family [1]. It is a vital food source for humans, providing essential nutrients like fat, protein, carbohydrates, and minerals (K, S, Ca, Mg, Cu, Na, and Fe) as well as vitamins (B1, B2, B6, and C) [2]. Additionally, eggplant possesses medicinal properties that can help to treat number of illnesses, including diabetes, asthma, bronchitis, otitis, arthritis, and cholera [3]. Magioli and Mansur, (2005) noted that eggplant tissue exhibits high morphogenetic potential, which is beneficial developmental studies biotechnological applications aimed at producing improved varieties, such as *in vitro* selection embryo rescue, genetic transformation, and somatic hybridization [4].

Tissue culture is an effective biotechnology method for producing callus, micropropagation, and root formation from plant [5]. The success of callus induction depends on numerous variables including plant genotypes, culture conditions, media composition, and explant selection [6]. Researchers are actively investigating and optimizing these parameters to enhance plant regeneration. Different explants respond variably to plant growth regulators (PGRs) in the culture media to callogenesis; highest amount of callus was produced from leaf and hypocotyle and on Murashige and Skoog media (MS) containing 0.5 mg l⁻¹ naphthalene acetic acid (NAA)+1 mg l⁻¹ BA [7, 8]. Additionally, a combination of 0.5 mg l⁻¹ NAA+ 2 mg l⁻¹ Benzyl amino purine (BAP) has shown high callus induction from epicotyle [9], hypocotyle [10] and leaf explants [11]. Foo *et al.*[12] emphasized that 0.05 mg l⁻¹ BAP combined with 2 mg.l⁻¹ NAA produced best callus form cotyledonary leaves, while 4 mg ml⁻¹ BAP produced best callus from hypocotyl after 18 days of culture. Conversely, Bhat *et al.* [13] found the best treatment for highest callus induction from cotyledon explant when using (8.88+1.14) μ M BAP + IAA respectively. The objective of this study is to identify the optimal explant and plant growth regulator (PGR) combination to generate superior calluses and promote rooting."

2. Materials and Methods

The experiments for this study were carried out in the tissue culture laboratory of the Department of Genetic Engineering at the Directorate of Agricultural Research, Ministry of Science and Technology

2.1. Seeds disinfection program

Under aseptic conditions inside laminar air flow cabinet, the mature seeds of *S. melongena* L. (cv. KEMER 27) were brought from Directorate of Agricultural Research and soaked in ethanol (70%) for 30 sec., followed by a rinse in sterile distilled demineralized water (DDW) for three times. Then washed with 70% Clorox (commercial bleach solution) for 20 min. and rinsed 4-5 times with sterilized Double Distilled water (DDW) to remove excesses sterilization solution effect [14].

2.2. *In vitro* germination

Ten seeds of *S. melongena* L. were planted in vitro to germination and obtaining seedlings in 4.4g.l⁻¹ of Murashige and Skoog media (MS) supplemented with 30g.l⁻¹ sucrose, agar-agar 7g.l⁻¹ and varying concentrations of gibberellic acid (GA₃) 0, 1, 2, and 4mg.l⁻¹. The pH was adjusted to the range of 5.7 to 5.8. All treatments were maintained in growth room at 24±1°C in alternate light/dark conditions (16/8 hrs. photoperiod) with 3000 lux of light intensity for 14 days.

2.3. Callogenesis

Hypocotyl and leaf explants were isolated from 14-day-old *in vitro* seedlings. The explants were subsequently inoculated under aseptic conditions in a laminar flow air cabinet on a solid MS medium supplemented with several combinations of PGRs to stimulate callus formation (Table 1). The cultures were kept in growth room at 16h light / 8h dark photoperiod and 24±1°C for 30 days.

Table 1: PGRs used for callogenesis.

Code	PGRs (mg.l ⁻¹)
T1	0.2 Kn. + 0.4 BA
T2	2 Kn. + 2 BA
T3	2 Kn.+ 2 BA + 2 IAA
T4	2 BA + 2 2,4-D
Kn.= kinetin, BA=Benzyl adenine, IAA=Indole acetic acid, 2,4-D=Dichlorophenoxy acetic acid	

2.4. *In vitro* root induction

For root induction, *in vitro* shoots measuring 4-5cm in length were excised from seedlings and cultured in MS medium supplemented with 1mg.l⁻¹ of indole butyric acid (IBA) and NAA in addition to control treatment (free hormone). All treatments were kept in growth room at 24±1°C in alternate light/dark conditions (16/8 hrs. photoperiod) with a light intensity of 3000 lux for 25 days.

2.5. Acclimatization

For successful acclimatization, *in vitro* rooted plantlets from previous stage were washed with DDW three times to remove any remaining agar and sucrose remains and transferred to plastic cup (ex-vitro environment) containing ½MS medium liquid (without sugar) and covered with transparent bag to maintained the relative humidity at 80±5% with temperature 28±1°C under 3000 lux of light intensity for 14 days (lab. conditions). The hardened plantlets were subsequently moved pots containing autoclaved mixture of peat-moss and soil (1:2) under plastic-house conditions for 28 days and calculated the plant survival rate.

2.6. Parameters Scoring

1. Germination Percentage(GP) (%): was calculated as follow:

$$GP (\%) = \text{Total seeds germinated} / \text{Total No. of seeds} \times 100$$

2. Germination Time (GT) (day).

3. Seedling Height (SH) (cm): was calculated by ruler.

4. Callus Induction(CI) (%): was calculated as follow:

$$CI (\%) = \text{No. of explants induced calli} / \text{total No. of cultured explants} \times 100$$

5. Callus Nature (CN): was recorded and graded as 3 for compact texture, 2 for friable texture and 1 for loose texture.

6. Callus Abundance (CA): was recorded and graded as +++ for plenty, ++ for moderate and + for poor.

7. Fresh and dry weights of callus (g): fresh weight (FW) determination by using electrical balance after drying at 45°C for 24h in oven to evaluate the dry weight (DW).

8. Relative Water Content (RWC)(%) : was calculated as follow:

$$(RWC)(\%) = [(FW - DW) / FW] \times 100$$

9. Root Induction(RI) (%): was calculated as follow:

$$\text{Root induction (\%)} = \text{No. of shoot induced root} / \text{Total No. of shoot} \times 100$$

10. Root Number / shoot (RN) (root).

11. Root Length (RL) (cm): was calculated by ruler.

2.7. Experiment design and statistical analysis

The experiment was structured using a Completely Randomized Design (C.R.D.) with five replicates for each parameter. Statistical analysis was performed using GenStat¹² software programs and means were compared using Duncan's multiple range test at 5% level.

3. Results and Discussion

3.1 Seed Germination

Table (2) illustrates a gradual and significant increase in germination % with rising concentration of GA₃ and the highest values were obtained 100% in medium supplemented with 2 and 4mg.l⁻¹ GA₃, compare with 32% at medium free of GA₃ (control). In contrast, the addition of GA₃ resulted in a significant decrease in germination time compared with the control which gave highest value 12.2 day. Concerning seedling height, 4 and 2mg.l⁻¹GA₃ affected positively on seedling height with 3.9 and 3.8 cm respectively compared with 1.2 cm at control treatment.

Table 2: Germination parameters under GA₃ concentration.

GA ₃ (mg.l ⁻¹)	GP(%)	GT(day)	SH(cm)
0	32c	12.2c	1.2c
1	88b	8.8b	2.7b
2	100a	5.2a	3.8a
4	100a	4.8a	3.9a

Means followed by the different letters at the same column are different significantly at 5% level according to Duncan's multiple range test.

The addition of GA₃ to MS media enhances seed germination and promotes cell division and elongation, resulting in improved eggplant seedlings development [17]. GA₃ is released from embryo during germination operation and stimulates specific genes for mRNA transcription by α -amylase that converts insoluble starch into soluble sugars which help in enhancement of germination [18]. Additionally, promotes seed germination by releasing coat dormancy, weakening endosperm and embryo cell growth [19]. According to Al-Darkazli *et al.* [20] GA₃ influences auxin metabolism, increase water accumulation, and cell membranes permeability. These results align with those of Neto *et al.* [21] found that GA₃ caused promoted germination and increase in seedling height of eggplant.

3.2 Callogenesis

Callus induction from hypocotyl and leave segments explants in response to various combinations of PGRs is presented in Tables 3 and 4. Treatments T3 (Kn.+ 2 BA + 2 IAA mg.l⁻¹) and T2 (2 Kn. + 2 BA mg.l⁻¹) were the most effective for callus induction from both hypocotyls and leaves explants with 100 and 96.0 % frequency respectively. In contrast, the combination (T1) treatment which represents (0.2 Kn. + 0.4 BA mg.l⁻¹) had a significantly lower result. The callus formed in the T3 combination was characterized by its compact nature, plenty abundance and white creamy to light green in color for both explants. Conversely, other PGR combinations were characterized by a fragmented or friable callus, a little abundance and spongy white. At the same time, no callus was observed from leaf explants in T1 (0.2 Kn. + 0.4 BA mg.l⁻¹). The results of the table 3 indicate that there were significant differences between the PGR combinations on callus fresh and dry weight which initiated from hypocotyle with highest average reached 504.2 and 53.9 mg at T3. The T2 gave 461.6 and 49.5 mg and T4 gave 415.7 and 44.3 mg respectively, while T1 recorded at which the lowest average of 115.2 and 13.6 mg respectively. Regarding leaf callus induction, the data in Table 4 revealed that T3 affected significantly and gave 429.3 and 45.7 mg for

fresh and dry weights respectively, with a notice that no significant differences were found among all treatments in related to RWC% characteristic for hypocotyls (table 3) while a superiority was found for T2, T3 and T4 as compare to T1 for callus induced from leaf (Table 4).

Table 3: Callus parameters for hypocotyle under PGRs combinations.

PGRs	CI (%)	CN	CA	FW(mg)	DW(mg)	RWC (%)
T1	80.0c	1	+	115.2 d	13.6d	88.20a
T2	94.0ab	3	+++	461.6 b	49.5b	89.27a
T3	100.0a	3	+++	504.2 a	53.9a	89.32a
T4	88.0b	2	++	415.7 c	44.3c	89.35a

Means followed by the different letters at the same column are different significantly at 5% level according to Duncan's multiple range test.

Table 4: Callus parameters for leaf under PGRs combinations.

PGRs	CI(%)	CN	CA	FW(mg)	DW(mg)	RWC(%)
T1	0c	-	-	0d	0d	0b
T2	78.0b	2	++	349.5b	38.2b	89.08a
T3	96.0a	3	+++	429.3a	45.7a	89.34a
T4	72.0b	1	+	267.9c	28.4c	89.28a

Means followed by the different letters at the same column are different significantly at 5% level according to Duncan's multiple range test.

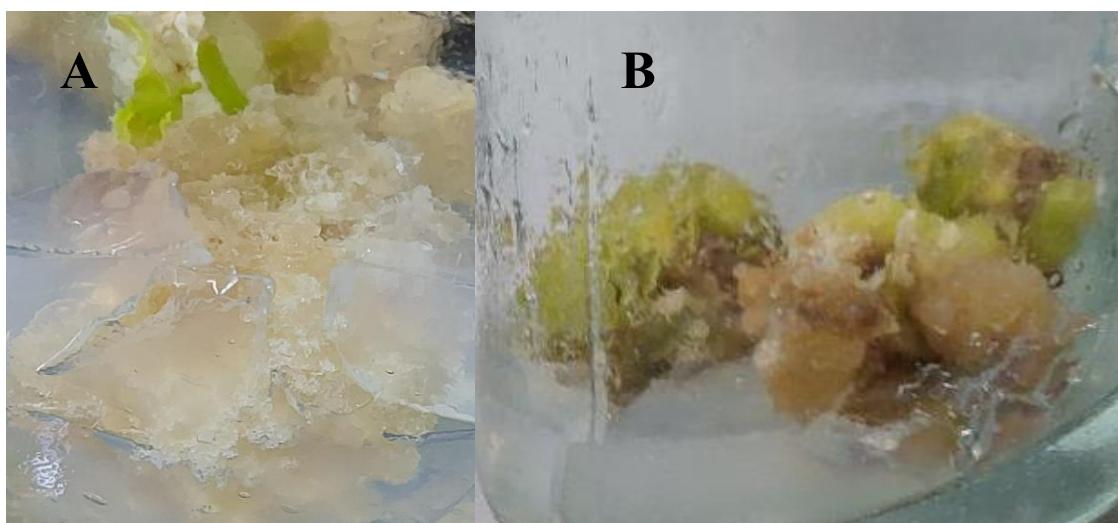


Figure 1 : Callus initiated from A: hypocotyls explants B: leaf explants in media T3 (2 Kn.+ 2 BA + 2 IAA mg.l⁻¹)

Auxins and cytokinins are commonly employed to stimulate callus formation at various concentrations. Our findings indicate that different combinations of PGRs were added to MS medium for *S. melongena* explants (hypocotyl and leaf), resulting in differences in the induction percentage, type, abundance, and color of the callus. Furthermore, present findings revealed that the interaction between exogenous hormones had a significant impact on the average fresh and dry weight of the induced callus of eggplant from explants treated with 2 mg l⁻¹ Kn+2 mg l⁻¹ BA+2 mg l⁻¹ IAA.

The type and concentrations of PGR are critical factors influencing callus formation and morphogenic responses of eggplant. Additionally, variations in callus and organ induction

may be a result of genotype or cultural conditions [22]. Sharma and Rajam, [23]; Mir, *et al.* [24]; Franklin *et al.* [25] successfully induced callus successfully from *S. melongena* using cotyledon and hypocotyl explants on MS media with NAA and BAP. Explants from 30day-old seedlings showed superior callus induction and regeneration compared to older plants, possibly due to genotype or cultural factors.

Some researchers have suggested that variations in explant responses could be attributable to physiological activity of the donor plant, potential of totipotency, content of internal hormone and metabolism, which all reflect in different physiological responses that vary depending on PGR combinations [26,27], However, others have noted that the leaf explant of eggplant produced the highest frequency of callus induction and fresh weight which they believe is due to its appropriate hormone content, tissue and genetic nature [12,13]. The findings of the present study align with those reported by previous researchers [11- 14].

3.3 *In vitro* root induction

The results show significant differences in root parameters at varying concentrations of IBA and NAA. Specifically, IBA exceeded NAA in terms of root percentage, numbers, and lengths of roots, achieving 83%, 6.75, and 3.87 cm, respectively, compared to NAA, which recorded lower values of 66%, 4.51, and 2.69 cm, respectively. Furthermore, the findings indicate that the control group (free of hormones) exhibited the lowest values for all root parameters compared to both IBA and NAA treatments (Table 5, Figure 2).

Table 5: Root parameters under PGRs(mg.l⁻¹).

PGRs	RI (%)	RN (root)	RL (cm)
control	41c	2.67c	1.37c
IBA	83a	6.75a	3.87a
NAA	66b	4.51b	2.69b

Means followed by the different letters at the same column are different significantly at 5% level according to Duncan's multiple range test.



Figure 2: Rhizogenesis of eggplant shoots in IBA, NAA and control treatments.

The induction of *in vitro* root formation depends on various factors including culture conditions, genotype, and PGRs, typically auxins like IBA, IAA, and NAA, either alone or in combination with different concentrations [28]. This process typically demands a substantial amount of auxin. The results of this study demonstrate that 1mg.l⁻¹ IBA is more effective than 1mg l⁻¹ NAA, while the control treatment (auxin free medium) generated the lowest rooting

formation. This could be attributed to the low levels of endogenous auxin in the rooted shoots. IBA is widely utilized for root induction in plant tissue culture systems due to its non-toxic nature over a broad range of concentrations and its slow rate of oxidation [29]. Additionally, it induces changes in many enzymes, nucleic acids, carbohydrates, and proteins in the rooting zone, thereby enhancing division and differentiation processes [30]. Numerous researchers have cited the positive role of IBA in rooting for eggplant such as Ray *et al.* [10] and Kaur *et al.* [11].

3.4 Acclimatization

The results of acclimatization under laboratory conditions indicated a plant survival rate was 77% (fig. 3A), while under plastic-house conditions recorded 90% (Fig. 3B). Cultivating plants put in $\frac{1}{2}$ MS aimed to reduce the salt concentrations in the rooting media, led to the emergence of the phenomenon of trophtropism, which stimulates an increase in the length of the roots in order to compensate for the deficiency. Additionally, the use of peat moss enhances the biological characteristics of the soil and , thereby increasing its fertility [31].



Figure3: Plantlets acclimatization: A: Lab. conditions B: Plastic –house conditions

Conclusion

GA₃ improves seed germination and seedling growth of *S. melongena*, with optimal callus from hypocotyle and leaf explants appeared during 30 days in combination of PGRs 2 mg l⁻¹Kn+2 mg l⁻¹BA+2 mg l⁻¹IAA. The second part of this research concentrated on studying *in vitro* root induction which is closely related to IBA. The regenerated plantlets can be acclimatized in pots filled with a mixed from mixture of peat-moss and soil (1:2) under plastic-house conditions.

Conflict of Interest: "The authors declare that they have no conflicts of interest."

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