INFLUENCE OF GROWTH REGULATORS ON CALLUS INDUCTION, GROWTH AND BIOMASS ACCUMULATION FROM DIFFERENT EXPLANTS OF LENTIL (*LENS ESCULENTA* MOENCH.)

INFLUENȚA UNOR REGULATORI DE CREȘTERE ASUPRA INDUCERII PROCESULUI DE CALUSOGENEZA ŞI ACUMULAREA DE BIOMASĂ A DIFERITOR EXPLANTE DE LINTE (*LENS ESCULENTA* MOENCH.)

MORARIU Aliona, CIOBOTARI Gh., DASCĂLU M.,CAULEȚ Raluca Petronela, ȚÂRDEA Gh.,
University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

Abstract. *Callus cultures were induced starting from excised small pieces of hypocotyl and cotyledon from plantlets obtained trough aseptic germination of Lens esculenta Moench ssp. microsperma seeds, cv Oana. Several combinations of different concentration of 2,4D, AIA and kinetin were tested for callogenesis intensity evaluation. On a medium containing a high level of auxin, a first primary callus was induced which was friable, unorganized and capable of somatic embryogenesis. This callus type was characterized by fast growth and high morphological variability. Biomass accumulation was determined after 4 weeks of subcultivation. A clear influence of growth regulator balance and combination was observed in promoting callus growth and morphogenesis. The highest biomass accumulation was observed on MS medium supplied with 1:2 auxine / citokinines ratio, but a highest mean of responsive explants percentage was on the medium supplied with 2:1 auxine /citokinines ratio.*

Key words: *Lens esculenta* Moench., callogenesis, biomass.

Rezumat. *Cultura de calus a fost inițiată pornind de la explante de hipocotil şi cotiledoane excizate de la plantule obținute din seminte de linte, Lens esculenta Moench ssp. microsperma, cv Oana, germinate aseptic. Pentru evaluarea intensității procesului de calogeneză s-au testat câteva combinații de 2,4D, AIA si kinetină în diferite concentrații. Pe mediul cu concentrații sporite de auxine calusul obținut a fost friabil, de culoare deschisă, cu potențial embriogen. Acest tip de calus s-a caracterizat printr-o creştere rapidă şi variabilitate morfologică mare. Acumularea de biomasă s-a determinat după 4 săptămâni de subcultivare. S-a constatat o influență clară a balanței fitohormonale asupra intensității calusigenezei şi a proceselor de morfogeneză. Cea mai mare acumulare de biomasă s-a constatat pe mediile suplimentate cu un raport auxine citochinine de 1:2, pe când cel mai mare procent de explante care au avut un raspuns morfogenetic a fost înregistrat la suplimentarea mediilor cu un rapotrt auxine /citockinine de 2:1.*

Cuvinte cheie: *Lens esculenta* Moench., calusogeneză, biomasă

INTRODUCTION

The lentil (*Lens esculenta* Moench) is an important seed legume widely cultivated in the Middle East, Southern Asia and throughout the tropical and subtropical regions, where it provides a large proportion of the dietary protein requirements. It also improves soil fertility by fixing atmospheric nitrogen, thereby providing an excellent break crop, profitable in its own right to the intensive cereal farmer. However, lentil production is threatened by many insects, diseases and weeds. Because of its potential usefulness for human consumption it is interested in biotechnological methods to improve this important plant. Development of an efficient regeneration system would substantially assist breeding of this crop for improvement. Tissue culture and regeneration studies on lentil are very restricted and there is a limited report on lentil regeneration when compared to other species. First report about lentil tissue culture is regeneration from cultured soot tips (Bajaj, 1979). This study is followed by culturing portions of shoot meristems and epicotyls on a medium containing kinetin and giberllic acid to induce the formation of callus tissue which is then regenerated shoots and rooted in a mist chamber to yield whole, fertile plants (Williams and McHughen, 1986). Polanco et al (1988) reported the influence of some growth regulators and explant type on callus and shoot formation. It was reported that, 2,4D induced callus formation in all explants, but no organ regeneration obtained from this calli. Callus production can be of interest in a crop improvement program as a propagation tool through organogenic and somatic embryogenic induction. Inductive callus lines are also useful for genetic manipulation practices such as transformation, protoplast isolation and fusion, and polyploid induction.

MATHERIALS AND METHODS

Oana varietie of lentil (*Lens culinaris* Medik.) develop by prof. Gh. Tardea and caracteryzed by high yield potential and proteine content were used in the present investigation. Seeds were first soaked in 70% ethanol for one minute and then they were surface sterilized with a 5 % hypoclorid solution for 20 min and there after washed with sterilized distilled water 3 - 4 times. The surface sterilized seeds were then cultured on 0.4% (w/v) water-agar medium and kept in the dark up to their germination in a growth room at 21 ± 2_C in the darknes.

Collected cotyledons and hypocotyls from germinating seeds (3-4 days old) were cut into pieces and were placed on MS medium supplemented with different concentrations of 2,4-D for callus induction. Induced calli were subcultured into fresh media after a 21-28-day interval for developing an organogenic nature. Watery, spongy, very compact, brown and dead portions of calli were discarded during every subculture. Friable, nodular calli were assumed potentially organogenic and were selected for maintenance and regeneration. As the carbon source 3% sucrose was used in all media. After adjusting the pH to 5.7 ± 0.01 prior to gelling with 0.8% agar (w/v), the medium was sterilized by autoclaving at 121 °C for 20 min (1.06 kg/cm-2). Sterilized medium was poured into 100-ml flask (40 ml of medium) for use.

A different kind of auxin and cytokinin-supplemented MS medium was used in the present investigation. All explants were cultured on MS medium with various concentrations of hormonal supplements presented in Table no.1. The culture vessels were incubated in the growth room under 16/8 hrs light/dark cycle at 25 ± 2 ^{o}C.

Table 1

Phitohormone type and concentration used in callus induction and growing experiment

No.	Variant	2,4D (mg/l)	AIA (mg/l)	K (mg/l)
1	A1	0	0	1
2	A2	0.5	0	1
3	A3	1	0	1
4	A4	2	0	1
5	A5	1	0	0
6	B1	0	1	0
7	B2	0	0.5	1
8	B3	0	1	1
9	B4	0	2	1

RESULTS AND DISCUSSIONS

In our study eight different medium in their growth regulator compositions, were employed to examine the callus induction potency of cotiledonary and hypocotyls explants. Callus induction results after 4 weeks of incubation (table 2) showed that each growth regulator combination except A1and A5 variants of medium gave high percentage of callus induction of both explants were we used. Callus was induced on medium containing 2,4-D, but not on auxines-free medium. The highest frequency of callus induction, 100%, was observed in MS medium containing 2:1 ratio from both explants. The proliferation efficiency of callus of hypocotyls explants was significantly higher than that of cotyledon explants for four to five weeks incubation of culture. The highest mean of responsive hypocotyls percentage was 100% in compare with highest mean of responsive cotyledons percentage when was 91%. 2mg/l^{-1} level of auxines was optimum for morphogenesis response induction on hypocotyls explants. Levels below this gradually decreased the frequency of callus induction. When we used cotyledon explants for callus induction we can observe the same reaction to the AIA supplement, but maximum mean of responsive explants at 1 mg/l concentration of 2,4D.

Each combination of plant growth regulations showed different calli formations. Explants incubated on medium with 1:2 ratio of auxine/ citockinines go into a callogenesis and develop a hard, compact and smooth callus, which is probably because of auxine influence on cell elongation. Medium with increased content of auxines showed a watery and very soft, friable calli development that is probably the first stages of embryogenesis.

Both the color and texture of the callus derived from the studied explants varied. Calli derived from hypocotyls were mostly friable and creamy in color (figure 1), with very few brownish exceptions. Cotyledon derived calli were mostly watery and pale brown and had less potential for further organogenesis. It was further observed that the presence of light affected callus induction and proliferation.

2,4-D is among the most widely used auxins for *in vitro* callus induction in a wide range of plant species. In our study, successful induction of potentially organogenic callus from hypocotyl and cotyledons was achieved using 2,4-D.

Table 2

Effect of different growth regulator concentrations on callus biomass accumulation from hypocotyls explants

No	Media variant	Explant no	% of explants	Initial mass of explants(g)	Biomass (g)	Relative biomass (%)
1	A1 0/1	0	0	0.22	0.31	40,91
2	A2 1/2	15	55	0.35	3.75	971,43
3	A3 1/1	21	70	0.27	1.37	407,41
4	A4 2/1	30	100	0.26	0.9	246,15
5	A5 1/0	7	23	0.21	0.42	100,00
6	B1 1/0	25	83	0.21	0.66	214,28
7	B2 1/2	29	98	0.1	0.51	410,01
8	B3 1/1	21	70	0.29	0.85	193,10
9	B4 2/1	30	100	0.12	0.4	233,33

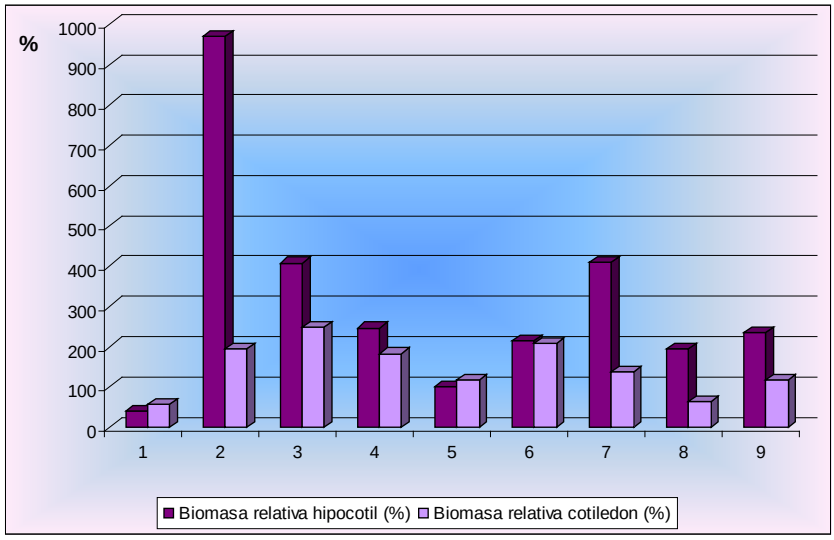

Fig.1. Effect of different growth regulator concentrations on callus biomass accumulation from hypocotyls and cotiledonary explants

Similar results were reported previously in this species (Leljak-Levanic et al., 2004). Moreover, Srivastava et al. (1989) obtained organogenic calli using a combination of BAP and NAA. We showed that friable and creamy calli derived from hypocotyl can be used to initiate organogenic calli. Calli induced from hypocotyl explants were larger in size than those from the cotyledon (table 3), and when transferred to a basal medium at low levels of PGRs it can promoted shoot organogenesis.

Table 3

Effect of different growth regulator concentrations on callus biomass accumulation from cotiledonary explants

No	Media variant	Explant no	% of explants	Initial mass of explants(g)	Biomass (g)	Relative biomass (%)
1	A1 0/1	0	0	0.09	0.14	55,55
2	A2 1/2	10	33	0.15	0.44	193,33
3	A3 1/1	21	84	0.15	0.52	246,66
4	A4 2/1	16	64	0.17	0.48	182,35
5	A5 1/0	2	10	0.11	0.24	118,18
6	B1 1/0	20	66	0.13	0.4	207,69
7	B2 1/2	26	87	0.16	0.38	137,5
8	B3 1/1	11	44	0.24	0.39	62,5
9	B4 2/1	27	91	0.22	0.48	118,18

In order to analyze the interaction of cytokinines tested with 2,4D and AIA, fresh weights were evaluated. Results of average calli weight in both explants after fourth week are given in fig. 1. According to these results the best responding medium was medium A2 and B2 for hypocotyls explants and A3 for cotiledonary eplants. Based on the results the maximum growth of callus was obtained from hypocotyls explants in MS medium amended with 2,4-D at 0.5 mg/l. and 1 mg/l K (table 2). We can observe an indirect dependence between increasing of 2,4D concentration above this level and biomass accumulation. The maximum callus growth of hypocotyls explants cultivated on the AIA supplied media was found also at 0.5 mg/l concentration of this phytohormone. We can also observe that hypocotyls was been found as a suitable explant to induce a hight biomass accumulation with auxins such as 2,4-D and AIA, and also with K among the cytokinins. Callus in 2,4-D supplemented medium was well developed, albino, spongy, and loosely arranged (figure 1). The moisture content of callus was high as compared to other auxins supplemented media. In AIA supplemented medium, the callus was pale, yellowish green in colour more friable, hard and granular (figure 1). Based on the obtained results, the high biomass was induced on only AIA supplemented media then only 2,4-D supplemented media. Exogenous application of auxin is indispensable for initiating callus formation of the tissue, but kinetin is not necessarily required. Kinetin serves to maintain the callus development, indicating that the stimulation

of callus growth due to exogenous auxin would presumably be mediated by the addition of kinetin to the medium.

CONCLUSIONS

1. Callus was induced on each medium containing 2,4-D and AIA, but not on auxines-free medium.

2. Despit the fact that callus appears in all growth regulators combination tested, the consistency was different and those was well developed, albino, spongy, and loosely arranged in 2,4-D supplemented medium and in AIA supplemented medium, the callus was pale, yellowish green in colour more friable, hard and granular.

3. Medium with increased content of auxines (2:1 ratio) gave the best callus quality (frible, loose, white and abundant).

4. The maximum growth of callus was obtained from hypocotyls explants in MS medium amended with 2,4-D at 0.5 mg/l. and 1 mg/l K.

REFERENCES

1. **Bajaj YPS, Dhanju M.S., 1979** - *Regeneration of plants from apical meristem tips of some legumes*. Current Sci. 48: 906–907
2. **Khanam R., 1994** - *Study of in vitro morphogenesis in lentil (Lens culinaris Medik).* M. Sc. thesis, Department of Botany, University of Dhaka, Bangladesh.
3. **Polanco M.C., Pelaez M.I., Ruiz M.L., 1988** - *Factors affecting callus and shoot formation from in vitro cultures of Lens culinaris* Medik. Plant Cell, Tissue and Organ Cult. 15(2): 175-182.
4. **Srivastava D.R., V. M. Andrianov, E.S. Piruzian, 1989** - *Tissue culture and plant regeneration of lentil (Lens culinaris Medik).* Plant Cell Rep. 8: 300-302.
5. **Williams D.J., McHughen A, 1986 -** *Plant regeneration of the legume Lens culinaris Medik. (lentil) in vitro.* Plant Cell, Tissue and Organ Cult. 7: 149-153.