In Vitro Culture and Effects of Explant Coculturing in Canola (Brassica napus L.)

Mehrabi.A.A[1*], M.omidi[2], B.E.Sayed Tabatabaie[2] and A.A.Sh.Boshehri[2]
1.M.Sc student of plant breeding 2.Assistance professors of Department of Agronomy and Plant Breeding, faculty of Agriculture, University of Tehran, Karaj, Iran.
*: Email: mehrabi_A@hotmail.com

Abstract

In order to investigate callus induction and regeneration in canola, hypocotyledonary explants from 6day seedlings, were cultured in MS medium complimented with 2mg lit^{-1} BAP and 1mg lit^{-1} NAA. Callus initiation was earlier in Syn1 and the most callus induction was in PF7045/91. Calli of Okapi and Colvert were greater than other genotypes. After sub culturing of calli in two regeneration media with different growth regulators, shoot regeneration in Regent * Cobra was more than others. ANOVA for callus volume showed that medium and genotype were significantly different, but embryogenesis was significant only for genotypes. In all of these procedures SLM.046 didn't response to hypocotyledon in vitro culture. In coculturing of different explants of Colvert genotype in modified MS (excluding Pyridoxin-HCl and Nicotinic Acid, and including 0.4mg lit^{-1} Thiamin-HCl, 2mg lit^{-1} BAP and 0.2mg lit^{-1} NAA) cotyledon-hypocotyle cultures had the best callus induction response and in all of the obtained calli, embryogenesis occurred. But the shoot regeneration was observed only in cotyledon culture. In this research, roots cultured with other explants, seriously influenced callus induction and differentiation of them. It seems that these effects are related to special substances in root explants that identification of these factors would lead to epigenetic variation in callus cultures.

Key Words: Canola (Rape seed), Tissue culture, Callus, MS medium, Co culture.

Interaction

Canola is belong to the first of plants which its breeding is done by composition of traditional and modern methods. In this plant the most invitro methods for tissue culture have been used successfully at the high spectrum of explants (5,6). In canola relation to genotype, medium compounds, the kind and the age of explants, have been reported different responses. To produce of embryo genous and non embryo geneous calli from different tissue of a plant or callus cultures with the same genotype, involve heterogeneity of internal physiological conditions in all of the cells compose them. By using of coculturing of different explants from a plant or calli of different plant species internal physiological, can be change. Identification of these factors can be lead to organizing of directed epigenetical changes in callus cultures.The purpose of this research is to investigate of canola different genotype responses to invitro culture and interaction between explants in the coculture situation.

Materials and methods

First experiment: 10 canola genotypes originated from Iran and Europe obtained from seed and seedling development institute of Karaj,Iran(Table.1).

Table1: Characteristics of canola genotypes used in experiment.

Growth Type	Origin	Genotype
Winter	Italy	**Hansen**
Winter	France	**Colvert**
Spring	Germany	**PF7045/91**
Spring	Italy	**GWC**
Winter	Iran	**Syn1**
Winter	Nether land	**Consul**
Winter	France	**Okapi**
Winter	Iran	**Regent* Cobra**
Spring	Australia	**Hyola42**
Winter	Germany	**SLM.046**

At the first, seed surface dis infection carried out by using of 70% ethanol alcohol for 1min and then Sodium Hypocholorite (Naclo with 2.5% active choler) for 15min. In order to explant providing, germination medium including **½ MS** (without plant growth regulator) with 3% sucrose, 8gr agar in PH=5.8 was made and then sterilized at the 121°C and 1atm for 20min.Seeds planted in this medium in 140 x140 x 20mm petri dishes at 16hr light/8hr darkness photoperiod and 25±1°C in growth chamber. Hypo cotyledonary explants from 6day seedlings of each genotype prepared and cultured in MS medium complimented with 2mg lit^{-1} BAP and 1mg lit^{-1} NAA in 90 x 90 x 10 mm petri dishes. Cultures were kept in absolute darkness and 25±1°C .To evaluate of the ability of callus induction of

genotypes, the time of culturing to callus initiation, callus induction percentage, callus volume and root generation percentage (4week after explant culturing) were recorded. Callus volume was ranked by means of Hooker & Niber's method. Obtained calli transferred to regeneration mediums including MS compounds with 0.1mg lit^{-1} NAA and 2mg lit^{-1} BAP and the second medium including MS with 0.2 mg lit^{-1}IAA and 2 mg lit^{-1} Kinetin. 4week later, the previous growth traits besides of shooting percentage and the state of somatic embryogenesis calli were evaluated.

Second experiment: In this experiment from 6day seedlings of Colvert genotype, cotyledon, hypo cotyledon and root explants were provided and then different composition as (1.Root 2.Cotyledon 3.Hypo cotyl 4.Root+Cotyl 5.Root+ Hypocotyl 6.Cotyl+ Hypocotyl 7. Root+ Cotyl+ Hypocotyl) cultured in modified **MS** (excluding Pyridoxin-HCl and Nicotinic Acid, and including 0.4$mglit^{-1}$ Thiamin-HCl) and complimented with 2$mglit^{-1}$ BAP and 0.2mg lit^{-1} NAA. After 4week all of previous traits and callus fresh weight were measured. Statistical analysis of data carried out by means of Minitab and SAS soft wares.

Results

First experiment: the first symptoms of tissue swelling and cell propagation were observed in cultured hypocotyls of Syn1, Okapi and Konsul genotypes. Callus initiation in Hansen and PF7049/91 genotypes occurred latter than others. In all genotypes except to SLM.046, callus initiation carried out from hypocot explants. ANOVA of investigated traits, connected to callus induction show a significance differences among genotypes. The highest rate of callus induction observed in Syn1, Hansen, Consul and PF7045/91 and the greatest calli observed in Okapi and Colvert. However the most of the produced calli came root generator and maximum root generation was observed in PF7045/91genotype(Table.2).

Table 2: Mean comparison of evaluated traits of hypocotyl culture

Root generation %	Callus volume (H-N scale)	Callus induction%	Callus initiation (Day)	Genotype
93.33 a	8.53 cd	93.33 a	13.00 c	**Hansen**
93.33 a	13.2 a	93.33 a	7.66 ab	**Colvert**
100.00 a	8.93b c	10.00 a	12.33 c	**PF7045/91**
66.67 b	10.87 abc	66.67 b	9.00 ab	**GWC**
93.33 a	9.67 abc	93.33 a	7.33 a	**Syn1**
80.00 ab	12.73 abc	80.00 ab	7.00 a	**Consul**
80.00 ab	13.87 a	80.00 ab	7.33 a	**Okapi**
80.00 ab	80.00 abc	80.00 ab	10.00 b	**Regent * cobra**
66.67 b	7.20 d	80.00 ab	7.66 ab	**Hyola42**
0.00 c	0.00 e	0.00 c	Without callus d	**SLM.046**

Produced calli from cultured hypocots divided to: 1-Yellow color non-embryogenous calli with tiny, watery and condensed cells. 2-Limpid, embryogenous calli with coarse elongated, fragile and brittle shape. 3-Middle state calli with root generating property (Fig.1).

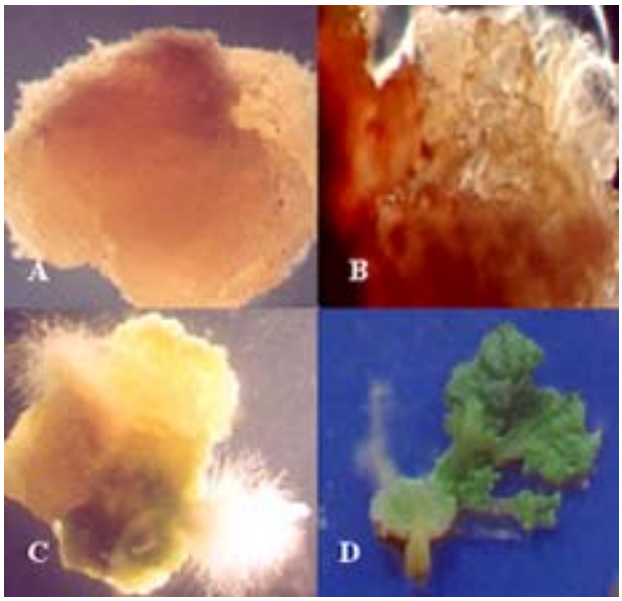

Fig.1: Different responses of callus culture in regeneration medium.
A: Non-embryogenous callus with watery and condensed shape. **B:** Embryogenous callus.
C: Root generated callus that almost complete shooting of it is impossible. **D:** Regenerated callus

After of transferring of obtained calli to two regeneration mediums, only in MS (2BAP+0.1NAA) medium shooting was occurred. ANOVA of callous volume and somatic embryogenesis in different genotype calli in two medium showed that genotype effect was significant, but different between two mediums was significant only for callus volume trait. In medium MS (2BAP+0.1NAA) the most of genotypes produced greater calli. The maximum of somatic embryogenesis was in Hansen and PF7045/91 and regeneration (at first shooting and then root generation) carried out in Colvert, PF7045/91 and Regent*Cobra genotypes, that Regent*Cobra have maximum regeneration (8.67%). In all of this stages SLM.046 genotype show no response to hypocotyl culture (Table3).

Table3: Mean comparison of evaluated traits in two regeneration mediums among different genotypes.

MS (2Kin+0.2IAA)		MS (2BAP+0.1NAA)			
Somatic Embryogenesis%	**Callus volume (H-N scale)**	**Regeneration%**	**Embryo genesis%**	**Callus volume (H-N scale)**	**Genotype**
31.67bc	13.06bcd	0.00d	41.67ab	14.52bcd	**Hansen**
21.67cd	13.58bc	6.33b	58.33ab	17.25ab	**Colvert**
71.67a	16.16a	3.23c	81.67a	18.33ab	**Pf7045/91**
20.00cd	12.33cd	0.00d	50.00ab	12.97cd	**GWC**
6.67de	11.11d	0.00d	8.33c	11.16cd	**Syn1**
15.00cde	11.92cd	0.00d	8.33c	11.86cd	**Consul**
36.67abc	15.25ab	0.00d	58.33ab	16.91ab	**Okapi**
48.33ab	15.16ab	8.67a	20.00bc	14.91bc	**Regent*Cobra**
75.00a	16.92a	0.00d	75.00a	19.44a	**Hyola42**
0.00e	0.00e	0.00d	0.00c	0.00e	**SLM.046**

Second experiment: In cocultuting of different explants of Colvert cotyl-hypocotyl show the best response to callus induction, as callus induction percentage, callus volume and fresh weight of callus was maximum in this composition. Embryogenesis was showed in all of the calli in cotyl-hypocotyl composition, but in calli obtained from other composition embryogenesis was not occurred. Direct shooting carried out in cotyledon explants that had been cultured separately. Exception of hypocotyl explants in all of explant compositions root generation was occurred (Table.4).

Table4: Mean comparison of evaluated traits in Co culturing of different explants .

Root generation%	**Callus fresh weight (mg)**	**Callus volume (H-N scale)**	**Callus induction %**	**Explant composition**
36.67a	50.33c	4.17cd	43.33c	**Root**
38.34a	436.67b	6.33bc	61.77bc	**Cotyledon**
0.00c	64.15c	4.5cd	70.00ab	**Hypo cotyledon**
23.33ab	74.33c	3.67cd	21.67de	**Root + Cotyledon**
20.00ab	54.27c	3.17d	15.00e	**Root+ Hypo cotyl**
33.33a	642.16a	14.00a	100.00a	**Cotyledon + Hypocotyl**
40.00a	56.73c	8.00b	20.00a	**Root+Cotyl + Hypocotyl**

The results of this investigation showed that culture of root explants near cotyledon and hypo cotyledon explants influence callus induction and differentiation of callus seriously (Fig.3).

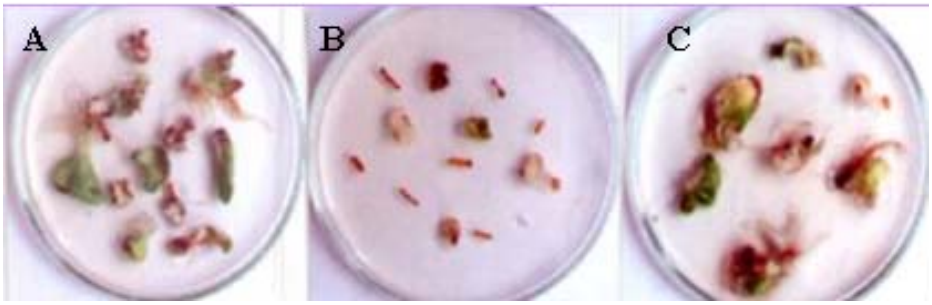

Fig.3: Observed responses from co culturing of different explants.
A: Culture of cotyl-hypocotyl explants near each other that showed the best responses relation to other compositions. **B:** Culture of root-cotyl-hypocotyl composition that root presence prevents callous induction and differentation in other explants. **C**: Culture of cotyledon separately. (These pictures have been taken 20 day after culture of explants).

Discussion

In this research observed different kind of embryogenous, non-embryogenous, organogenous and regenerated calli and their differences among different genotypes are related to serious correlation of this variation with genotype and the kind of cultured explants. On the other hand the presence of root generated calli in which regeneration is impossible, is related to presence of the cells which their evaluation in this stage has been ceased, that which of them have different genetic control and this is very important in this studies that how to turn and off the keys of these controls, but hitherto there are no explicit answer by which to determine the transition path of the callus cultures, moreover rapid disappearing of regeneration potential in this cultures is the major problem for the tissue culture researchers(2).

In callus culture of canola different genotypes, low shoot and root generation by side of genetic factors can be attributed to physiological factors. Identification of factors that are effective on the recovery of the canola callus culture situation needs more investigations. Extra stress to the cells cultured in canola callus culture through of mechanical wounds and the other hand medium external auxcin cane lead to induce the ethylene production by living cells.The reports of Shi. Shuwen(1998), Burrnet(1994), Hachy(1991) and sethi(1990)showed that the use of ethylene inhibitors such as AgNo3 and Amino Ethoxy Glycine cause to increase the percentage of regeneration of cotyledon and hypo cotyledon explants three time more than before using them. Ethylen production in response to stress exerted to cultured cells is apparently a secondary messenger (1). The results observed in second experiment show that decreasing of callus induction and regeneration of different explant composition of due to special substances in young root explant, that identification of these factors would lead to takeover in developing of callus culture state of canola. Beside this, the results of this investigation can be used for allelo patical, allelochemical, protoplast cultures, selection for mutant cells and to induce or control of somaclonal variation studies.

References

1.Arteca, R.N. 1996. Plant Growth substance (Principles and Application). Chapman & Hall. 331 pp.

2. Baghban, B .1992.Plant regeneration from corn and wheat in tissue culture and investigation of interaction between their calluses in coculturing situation. M.Sc thesis. Tehran University. Faculty of agriculture. Department of plant breeding.

3. Burrnet, L., M. Arnoldo., s. yarrow, B .Huang. 1997. Enhancement of shoot regeneration from Cotyledon explants of Brassica rapa ssp oleifera through pre treatment with auxin and cytokinin and use of ethylen inhibitors. Plant cell, Tissue and organ culture. 37: 253-256.

4. Hachy. J.E, K.K. sharma & M.M.Molony. 1991. Efficient shoot regeneration of Brassica Compestris using Cotyledon explant culture. Invitro. Pl. cel. Rep. 9:547-554.

5. Sethi. U, A .Basa & S.J. Mukherjee. 1990. Role of in hibitors in the induction of differentation in callus culture of Brassica, Da tura and Nicotina. Pl. cell. Rep. &: 598-608.

6. Shi. Shuwen. et al. 1998. A study of the techniques of hypo cotyl explants culture for high frequency shoot regeneration of rape seed. Chinese jornal of oil crop science. 20:2, 1-6. In CAB Abstracts 1999.