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Effects of light and different plant growth regulators on induction of callus growth in rapeseed (*Brassica napus L.*) genotypes

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Abstract

The present work describes the effects of winter and spring genotypes of rapeseed (*PF7045/91*, *Okapi* and *Opera*), explants (cotyledons and hypocotyls), light, different kinds and concentrations of auxins [Naphthylacetic acid (NAA) and 2, 4-dichlorophenoxyacetic acid (2, 4-D)], presence of cytokinin [benzyladenine (BA)] and different concentrations of micro and macro elements (MS and 1/2MS) on callogenesis in rapeseed (*Brassica napus L.*). First of all, explant sections were cultured in different combinations of media and plant growth regulators, in dark or light for 10 days. After 10 days, calli fresh weight and root formation were evaluated. When the effects of plant growth regulators on callus color were investigated, it was revealed that auxins had an inhibitory effect on chlorophyll formation, whereas cytokinin tended to promote it. The results also showed that 2, 4-D when combined with cytokinin (BA), callogenesis and cell division were stimulated faster and better in rapeseed cultivars whereas NAA (with BA or without it) stimulated root formation and rhizogenesis. Also, in most cases, calli fresh weight in winter genotypes of *Opera* and *Okapi* was higher than spring genotype of *PF7045/91*. Likewise, MS medium was more effective on callogenesis than 1/2MS medium. In all cultivars and explants (especially hypocotyl sections) light stimulated callus growth, inhibits root growth and in cotyledon derived calli increased browning. The results of this study suggested that using cotyledon sections as explants under dark conditions would be more beneficial for callogenesis, but for hypocotyl sections light conditions is preferred.

Keywords: callus induction, rhizogenesis, embryogenic clamps, callogenesis, rapeseed, plant growth regulator.
Abbreviations: MS Murashige and Skoog's medium; BA N^6-benzyladenine; BAP 6-Benzylamino-purine, NAA 1-naphthylacetic acid; 2, 4-D 2, 4-dichlorophenoxyacetic acid, PGRs plant growth regulators, CK Cytokinin, IAA indole-3-acetic acid, TDZ Thidiazuron.

Introduction

Rapeseed (*Brassica napus L.*) is a crop generally grown for oil or bio-fuel production and also for the high protein seed residue that can be used as animal feed after oil extraction (Rossato et al. 2001). During the last two decades its cultivation has increased enormously and now it is the second largest contributor to the world supply of vegetable oil (Ghnaya 2008). Thus, it is not surprising that in the recent years many researchers focused on its genetic improvement and like many other plants, plant tissue culture is a technique which is used widely by them. Usually the first step in plant tissue culture is callus induction. Callus is an amorphous tissue consisting of dedifferentiated, unorganized cell masses (George et al. 2008). The cells of callus are somehow parenchymic in nature. A typical plant callus will undergo three stages of development (George et al. 2008). The first stage is the induction of cell division (George et al. 2008). The second stage is dedifferentiation, which is a period of active cell division during which differentiated cells of the explants lose any specialized feature (George et al. 2008). Finally the last stage is the period during which cell division decreases or ceases and cellular differentiation increases within the callus (George et al. 2008). Depending on features like color, appearance, degree of compaction and morphogenetic potential there are different strains of callus even from a single explant (George et al. 2008). Many parameters like explants, species, cultivar, plant growth regulators and light affect callus induction, callus growth and callus quality for regeneration. Among plant growth regulators, auxins and cytokinins (CKs) are widely used for callus induction. Although all normal plant cells can produce both auxin and cytokinins but generally speaking, young shoot organs are the major sites for auxin (IAA) production and root tips are major sites of Ck synthesis in plants(Aloni et al. 2006). Auxins and CKs seem to be necessary for plant cell division. In plant tissue culture auxins are broadly used for callus induction. Auxins are involved in cell division, cell elongation, vascular tissue differentiation, rhizogenesis and root formation, embryogenesis and inhibition of axillary shoot growth (Chawla 2002; George et al. 2008; Park et al., 2010). It seems that auxins cause DNA to become more methylated than usual and this might be necessary for the reprogramming of differentiated cells and make them beginning division (George et al., 2008). The auxin commonly used for callus induction is 2, 4-D, but NAA and IAA are also used. CKs are derivatives of Adenine and seem to be required to regulate the synthesis of proteins which are

Table 1. Media type, PGRs and the concentrations of these PGRs used in this study.

Media No.	Media type	PGRs and concentrations used
M1	MS	NAA 1 mg l^{-1}; 2, 4-D 1 mg l^{-1}; BA 1 mg l^{-1}
M2	1/2MS	NAA 1 mg l^{-1}; 2, 4-D 1 mg l^{-1}; BA 1 mg l^{-1}
M3	MS	NAA 1 mg l^{-1}; BA 1 mg l^{-1}
M4	1/2MS	NAA 1 mg l^{-1}; BA 1 mg l^{-1}
M5	MS	2, 4-D 1 mg l^{-1}; BA 1 mg l^{-1}
M6	1/2MS	2, 4-D 1 mg l^{-1}; BA 1 mg l^{-1}
M7	MS	NAA 1 mg l^{-1}; 2, 4-D 1 mg l^{-1}
M8	1/2MS	NAA 1 mg l^{-1}; 2, 4-D 1 mg l^{-1}
M9	MS	NAA 1 mg l^{-1}
M10	1/2MS	NAA 1 mg l^{-1}
M11	MS	2, 4-D 1 mg l^{-1}
M12	1/2MS	2, 4-D 1 mg l^{-1}
M13	MS	NAA 2 mg l^{-1}
M14	1/2MS	NAA 2 mg l^{-1}
M15	MS	2, 4-D 2 mg l^{-1}
M16	1/2MS	2, 4-D 2 mg l^{-1}
M17	MS	NAA 2 mg l^{-1}; BA 1 mg l^{-1}
M18	1/2MS	NAA 2 mg l^{-1}; BA 1 mg l^{-1}
M19	MS	2, 4-D 2 mg l^{-1}; BA 1 mg l^{-1}
M20	1/2MS	2, 4-D 2 mg l^{-1}; BA 1 mg l^{-1}

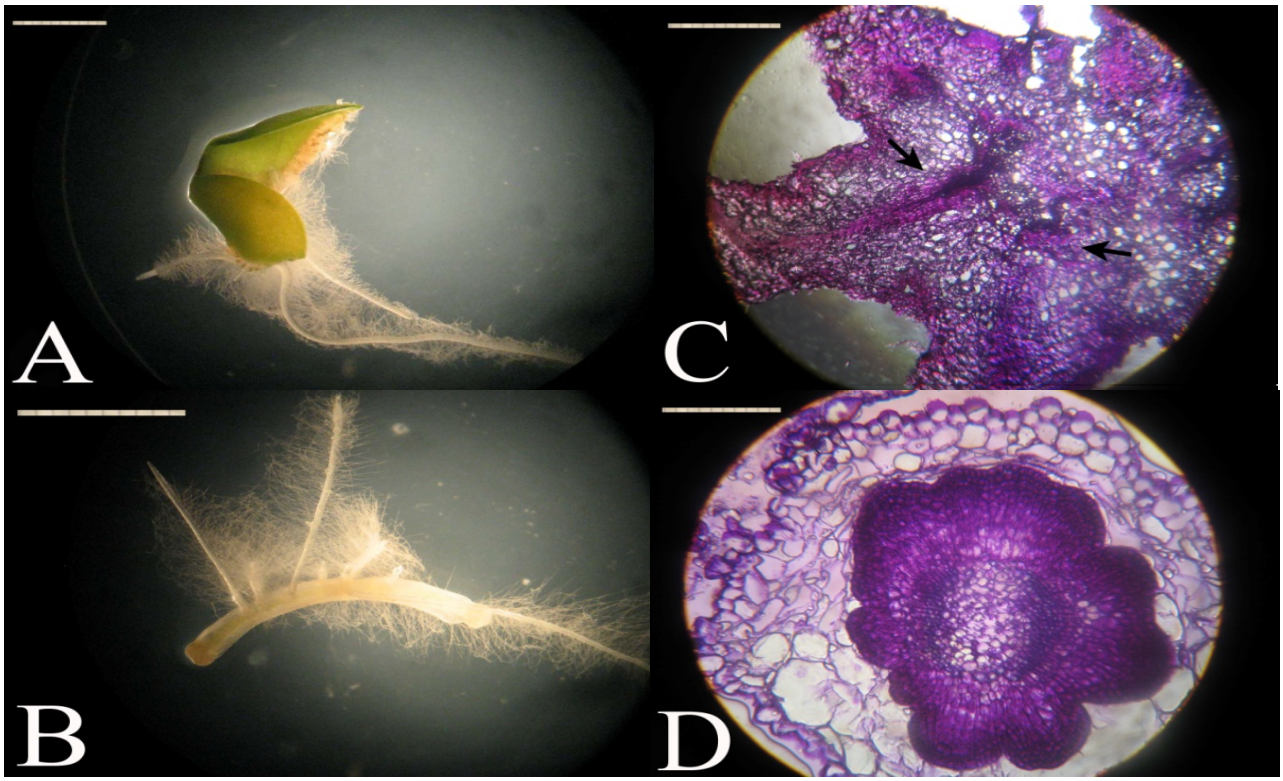

Fig 1. Root formation in cotyledon (A) (Opera, M3, light) and hypocotyl (B) (Okapi, M10, dark) sections (The scale bar for both pictures is 5 mm). (C) Microscopic view of roots formed on hypocotyl sections (Okapi, M10, dark) (The scale bar for this picture is 160 µm). (D) Changes in vascular tissue during root formation (Okapi, M14, hypocotyl, light) (The scale bar for this pictures is 100 µm).

involved in the formation and function of mitotic spindle apparatus (Chawla 2002; George et al., 2008). They are also required for adventitious shoot formation and stimulation of cell division (Chawla 2002; George *et al*., 2008). It should be mentioned that it seems callus tissues in which cells divide in media without CK, are able to produce their own natural CKs (George et al., 2008). Generally, CKs used in plant tissue culture are BA, BAP, zeatin, kinetin and more recently, TDZ. In sweet potato TDZ or BAP was not recommended for shoot development, however, they were useful for callus production (Alam et al., 2010).Light controls plants growth and development mainly in two ways: photosynthesis and photomorphogenesis. In plant tissue culture three aspects of photoenvironment mostly influence *in vitro* growth and morphogenesis; these are: wavelength, flux density and photoperiod. As a number of researchers mentioned light had significant effect on callus growth and morphogenesis, inhibition of axillary shoot proliferation and induction of specific enzyme activity which are concerned with the formation of some flavonoid glycoside secondary products (George et al. 2008). By now three kind of photoreceptors known as phytocrome (red and far red detector), cryptochrome (blue and UV-B detector) and phototropin (blue and UV-A detector) are thought to be involved in plant development (Ascencio-Cabral et al., 2008). There are some reports on the involvement of light on phytohormone synthesis, for example it has been shown that CK biosynthesis is stimulated to occur in red light, but is prevented when far red is used (George et al., 2008). Although the effects of these photoenvironment aspects had been studied by different scientists, our knowledge about the effect of photoenvironment on *in vitro* growth and morphogenesis is still limited.In the present study the effects of different media (different plant growth regulators and different concentrations of these plant growth regulators in MS and 1/2MS basal media), cultivar, explant and light on callus induction and callus weight in rapeseed (*Brassica napus* L.) genotypes were studied. For this reason many treatments were applied simultaneously. Generally, the aim of this study is to introduce a new protocol of callus induction of rapeseed by considering a set of nearly total factors which would influence the results. Moreover, a well-established protocol of callus induction is needed for further studies aimed to examine somatic embryogenesis of this plant.

Materials and methods

Plant materials

Three genotypes of rapeseed (*Brassica napus L.*) were used in this study; these were the winter cultivars *Okapi* and *Opera* and the spring cultivar *PF7045/91*. These seeds were given by the Oilseeds Research Department, Seed and Plant Improvement Institute, Karaj, Iran. They were harvested in 2007.

Chemicals

The ingredients of MS basal medium, BA (N^6-benzyladenine), 2, 4-D (2, 4-Dichlorophenoxyacetic acid), NAA (1-Naphthylacetic acid), Crystal Violet and Safranin were products from Merck KGaA, Germany.

Culture conditions

Seeds were surface sterilized with ethanol 70% for 1 minute followed by sterilizing with 2.5% sodium hypochlorite for 15 minutes. Then they were washed 3 times in sterile distilled water and germinated in a jar containing MS medium without vitamins, myo-inositol and PGRs with 1.5% sucrose and 0.8% agar at a destiny of 10 seeds per jar. These jars were then cultured in dark at 25^0C for 3 days and after that, they were transferred to fluorescent light (3000 Lux) generated by fluorescent lamps (Day light, 20W, 511, PARS, IRAN) at 25 ^{0}C and left to grow for 7 more days. After this time, the cotyledon and hypocotyl sections were transferred to 20 different media (Table 1). These media also contains 3% sucrose and 0.5% agar-agar. The pH was adjusted to 5.8.

Glass Petri dishes (12 cm) containing these media and explants were then cultured in either dark or fluorescent light (3000 Lux) generated by fluorescent lamps (Day light, 20W, 511, PARS, IRAN) at 25^0C for 10 days. Each Petri dish contains 5 pieces of either cotyledon (each cotyledon was cut transversely into two equal pieces) or hypocotyl (1 cm length) sections. After 10 days the induced calli were weighted (fresh weight) and the frequency of calli and root formation were calculated.

Histological observations

Two explant samples of cultivar *Okapi* were taken from each replicate for histological studies. These were fixed with F.A.A. [40% formaldehyde (10ml)/Acetic acid (glacial) (3ml)/50%ethanol (87ml)] (Sanderson 1994). The samples were then dehydrated through an ethanol series (from 30% to 100%) and embedded in paraffin. These samples were cut transversely with a rotary microtome (section thickness and knife angle were 7µm and 11^o respectively). Finally they were stained with Safranin (1 hour) and Crystal Violet (2 minutes), and photographed with an Olympus microscope coupled with a digital camera.

Data analysis

Treatments consisted of three cultivars (*PF7045/91*, *Opera* and *Okapi*), two explants (hypocotyls and cotyledons), two photoenvironments (light and dark) and twenty media (Table 1). For callus fresh weight the mean of five pieces in each Petri dish were calculated. These were arranged in a factorial experiment as completely randomized design. Each treatment combination was replicated three times. Statistical analyses were done using SAS (version 9.00) analysis of variance procedures (Table 2). Means of main effects interactions were compared by Tukey's Honestly Significant Difference Test of MSTATC software (Table 3, 4 and 5).

Results and discussion

All factors (different media, explants, cultivar and light) had significant effects on callus weight, not only individually but also when combined with each other (Table 2). The effects of mentioned parameters are explained as follow:

Callus formation

In cotyledon derived explants, newly initiated callus was creamy in the dark and mostly green in the light. In these explants, newly initiated callus in the media M13, M14, M15 and M16 with double concentration of NAA and 2, 4-D (2 mg l^{-1}) was very light green and creamy, respectively, even in the light (data not shown). This can be explained by the inhibitory effects of auxins on chlorophyll formation (George et al., 2008). It is likely that very light green media contain-

Table 2. Analyses of variance for the effects of cultivar, explant, light and media on callus weight.

Source of variation	DF	Mean Square
Cultivar	2	0.0364**
Explant	1	1.3330**
Light	1	0.1612**
Media	19	0.0347**
Cultivar*Explant	2	0.0086**
Cultivar*Light	2	0.0048**
Cultivar*Media	38	0.0035**
Explant*Light	1	0.0124**
Explant*Media	19	0.0109**
Light*Media	19	0.0036**
Cultivar*Explant*Light	2	0.0023*
Cultivar*Explant*Media	38	0.0013**
Cultivar*Light*Media	38	0.0019**
Explant*Light*Media	19	0.0017**
Cultivar*Explant*Light*Media	38	0.0020**
Error	480	0.0002
Total	719	-

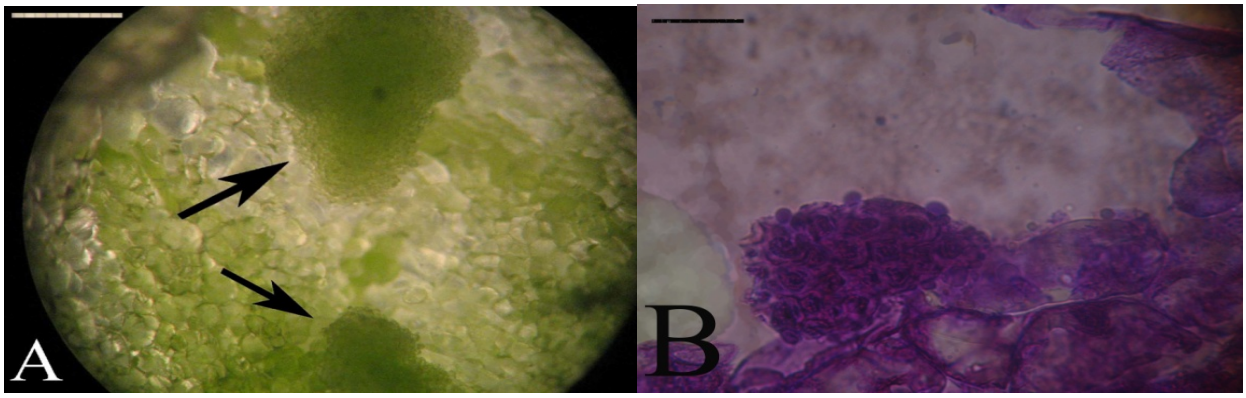

Fig 2. (A) Embryogenic clamps (black arrows) formed on cotyledon-derived calli of cultivar PF7045/91, in media M18 in the dark (The scale bar is 1mm). (B) The same embryogenic clam under the microscope (The scale bar for this picture is 50 µm). Small, compact cells with small vacuoles and large nucleus can be recognized easily.

ing NAA was due to the fact that NAA is less inhibitory on chlorophyll formation than 2, 4-D. The same thing happened in the media M17, M18, M19 and M20, but in these cases a color of very pale green was also observed which can be explained by the presence of BA, a kind of CK. CKs tend to promote chlorophyll formation (George et al., 2008). These were also the case in hypocotyl derived explants but since the hypocotyl contained less chlorophyll in the first place than the cotyledon, in the hypocotyl derived explants the range of color was from very pale green in the light to creamy in the dark. Application of 2,4-D was very effective on callus formation, especially when combined with cytokinin (BA). A proper combination of 2,4-D and kinetin promoted the callus initiation in cotton (Michel et al., 2008). The most recommended media for callus induction and growth in hypocotyl and cotyledon sections of rapeseed is B_5 or MS basal medium, containing 1mgl^{-1} 2,4-D (M11) (Jonoubi et al., 2005). But according to the results of this experiment (Table 3, 4 and 5) this protocol can be improved by adding CKs (here BA). In most cases, when it comes to micro and macro elements' concentrations, MS medium was better for rapeseed callus growth compared to half strength MS (1/2MS) (Table 3, 4 and 5). Higher level of exogenous auxin and addition of BA increased the difference between MS media but the differences were not statistically significant (Table 3, 4 and 5). The only exception was in cultivar *Opera*, in which contyledon derived calli in the light, M2 medium was significantly better than M1.

Callus weight in genotypes

Averaged overall, the highest fresh weight of calli derived from explants after 10 days was detected in cultivar *Opera* followed by *Okapi* and *PF7045/91* (data not shown). Also significant differences were detected in calli fresh weight among cotyledon and hypocotyls derived calli. Averaged overall, the mean of cotyledon derived calli was 0.1076 gr, whereas it was 0.0216 gr for hypocotyl derived calli. In cotton, callus inititation was genotype dependent and among ten genotypes, only one of them had the best callogenosis response (Michel et al., 2008). Interestingly, when the concentrations of 2, 4-D doubled (2 mgl^{-1}) (M19 and M20), callus weight didn't increase but even decreased in most cases. The only exception was cotyledon derived calli of cultivar *Opera* in M19 and M20 which was higher than those in M5 and M6. These differences may be due to differences

Table 3. Effects of explants, photoenvironment and different media on calli fresh weight (gram) in cultivar *PF7045/91*; values represent the mean of three replicates. Means followed by the same letter are not significantly different (Tukey's Honestly Significant Difference Test, Probability level= 0.05). Bold numbers show the highest mean in each explant and photoenvironment condition, the underlined numbers show the best conditions for calli induction and growth in each explant and photoenvironment condition.

PF7045/91	Cotyledon-Light	Cotyledon-Dark	Hypocotyl-Light	Hypocotyl-Dark
M1	0.1543^{bcd}	$\underline{0.1083^{bcdefghijk}}$	$0.05333^{lmnopqrst}$	0^{u}
M2	0.1597^{b}	$0.09567^{efghijklm}$	0.03233^{pqrstu}	0^{u}
M3	0.13^{bcdefg}	$0.1040^{defghijkl}$	$0.04567^{mnopqrstu}$	0.0076^{tu}
M4	0.1567^{bc}	$0.1263^{bcdefgh}$	$0.05367^{lmnopqrst}$	0^{u}
M5	$\underline{0.2307^{a}}$	$\underline{0.1063^{cdefghijk}}$	$\underline{0.05700^{klmnopqrst}}$	0^{u}
M6	$0.1203^{bcdefghi}$	$0.09233^{efghijklmn}$	0.03333^{pqrstu}	0^{u}
M7	$0.06333^{jklmnopqrs}$	$0.06167^{jklmnopqrs}$	0^{u}	0.009^{tu}
M8	$0.04500^{mnopqrstu}$	$0.04633^{mnopqrstu}$	0.03167^{pqrstu}	0^{u}
M9	$0.08633^{ghijklmno}$	$0.04333^{nopqrstu}$	0.02433^{qrstu}	0.02233^{qrstu}
M10	$0.03833^{opqrstu}$	$0.06567^{jklmnopqrs}$	0.02167^{rstu}	0.02133^{rstu}
M11	$0.08800^{fghijklmno}$	$0.06367^{jklmnopqrs}$	0.009^{tu}	0^{u}
M12	$0.04933^{mnopqrstu}$	$0.05367^{lmnopqrst}$	0.007^{tu}	0^{u}
M13	$0.07867^{ghijklmnop}$	$0.04567^{mnoprstu}$	0.02233^{qrstu}	0^{u}
M14	$0.05133^{mnopqrstu}$	$0.06833^{ijklmnopqr}$	0.02233^{qrstu}	0^{u}
M15	$0.07400^{ijklmnopq}$	$0.03867^{opqrstu}$	0.01567^{stu}	0^{u}
M16	$0.06300^{jklmnopqrs}$	$0.04667^{mnopqrstu}$	0^{u}	0^{u}
M17	$0.1093^{bcdefghij}$	0.1413^{bcde}	0.02133^{rstu}	0^{u}
M18	$0.09500^{efghijklmn}$	$0.04400^{mnopqrstu}$	0.025^{qrstu}	0.03067^{pqrstu}
M19	0.1397^{bcdef}	$0.07433^{hijklmnopq}$	0.02833^{pqrstu}	0^{u}
M20	$0.05167^{mnopqrstu}$	$0.07267^{ijklmnopqr}$	0^{u}	0^{u}

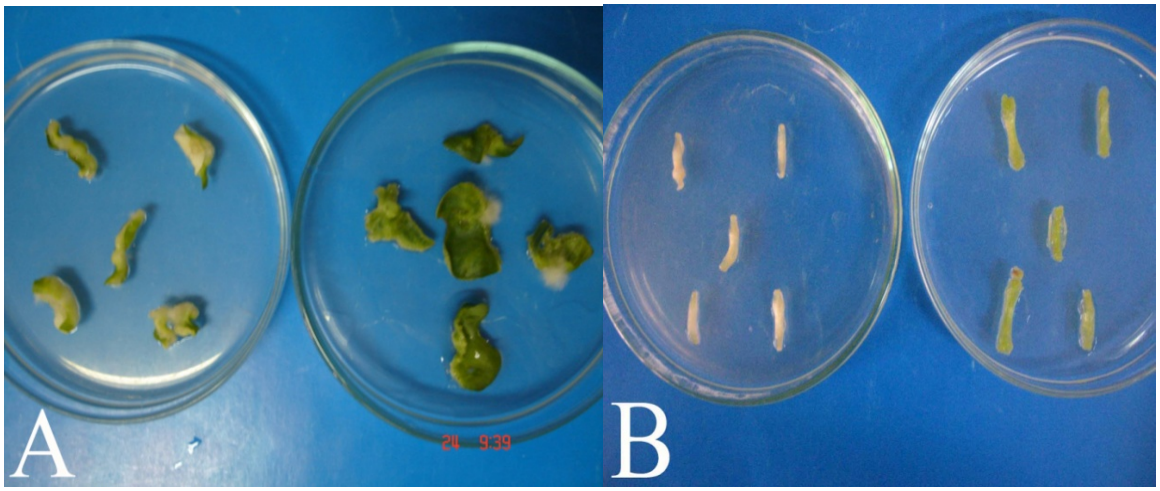

Fig 3. Calli weight and size in the dark and light. (A) Calli formed from *Opera* cotyledons which were in media M2, the left Petri dish was put in the dark and the right one in the light. (B) Calli formed from *Opera* hypocotyls which were in media M6, the left Petri dish was put in the dark and the right one in the light.

in cell's sensitivity to plant growth regulators, their capacity to dedifferentiate and/or the endogenous concentrations of plant growth regulators within these explants.

Rhizogenesis and Embryogenesis

In lower concentrations, auxins induced rhizogenesis and root formation, but in higher concentrations they induced callus formation (Chawla, 2002). In this experiment this was also the case for the weak (NAA) and strong (2,4-D) synthetic auxin. In other words, NAA induced rhizogenesis and root formation (especially in lower concentrations) whereas 2, 4-D induce callus formation. Those media containing NAA, whether with BA or without it (Table 1) exhibited a high frequency of rooting in both cotyledon and hypocotyl derived calli (Fig. 1), but when NAA is combined with 2,4-D no rooting or rhizogenesis were observed. Moreover, using higher concentration of 2,4-D (2 mgl^{-1}), resulted in low rhizogenesis and root formation. The effects of NAA on root formation and rhizogenesis was reported (Julliard et al., 1992). In these media, calli from both cotyledon and hypocotyl explants formed embryogenic clamps (Fig. 2). Embryogenic clamps are a mass of small, compact cells with a protein-rich cytoplasm, small vacuoles and large nucleus

Table 4. Effects of explants, photoenvironment and different media on calli fresh weight (gram) in cultivar *Okapi*; values represent the mean of three replicates. Means followed by the same letter are not significantly different (Tukey's Honestly Significant Difference Test, Probability level= 0.05). Bold numbers show the highest mean in each explant and photoenvironment condition, the underlined numbers show the best conditions for calli induction and growth in each explant and photoenvironment condition.

Okapi	Cotyledon-Light	Cotyledon-Dark	Hypocotyl-Light	Hypocotyl-Dark
M1	0.1573^{ef}	0.1420^{fghi}	0.02667^{tuvwx}	0^{x}
M2	$0.1170^{fghijkl}$	$0.09600^{hijklmn}$	$0.03433^{rstuvwx}$	0^{x}
M3	0.2673^{a}	0.2147^{bc}	$0.08400^{jklmnopqr}$	$0.04200^{pqrstuvwx}$
M4	0.2510^{ab}	0.1483^{efg}	$0.06967^{lmnopqrstu}$	$0.04333^{opqrstuvwx}$
M5	$\underline{0.2117}^{bcd}$	$\underline{0.1983}^{cde}$	$\underline{0.06133}^{mnopqrstuv}$	0.02900^{stuvwx}
M6	0.1350^{fghij}	0.1620^{def}	$0.04400^{nopqrstuvwx}$	0^{x}
M7	$0.08133^{klmnopqr}$	$0.04400^{nopqrstuvwx}$	0.005667^{x}	0^{x}
M8	$0.06233^{mnopqrstuv}$	$0.04300^{opqrstuvwx}$	$0.03600^{qrstuvwx}$	0^{x}
M9	$0.07700^{klmnopqrst}$	$0.07700^{klmnopqrst}$	0.02700^{tuvwx}	0.01400^{vwx}
M10	$0.09500^{hijklmno}$	$0.06033^{mnopqrstuvw}$	$0.03400^{rstuvwx}$	0.02167^{uvwx}
M11	$0.1153^{fghijkl}$	$0.04367^{opqrstuvwx}$	0^{x}	0^{x}
M12	$0.09033^{ijklmnop}$	$0.04700^{nopqrstuvwx}$	0.02233^{uvwx}	0^{x}
M13	0.1230^{fghijk}	$0.06867^{lmnopqrstu}$	0.01967^{uvwx}	0.008667^{wx}
M14	$0.08700^{jklmnopq}$	$0.04800^{nopqrstuvwx}$	0.02000^{uvwx}	0.01500^{vwx}
M15	$0.06967^{lmnopqrstu}$	$0.04733^{nopqrstuvwx}$	0^{x}	0^{x}
M16	$0.04433^{nopqrstuvwx}$	$0.04567^{nopqrstuvwx}$	0^{x}	0^{x}
M17	0.2877^{a}	$0.1100^{fghijklm}$	$0.04233^{pqrstuvwx}$	$0.04200^{pqrstuvwx}$
M18	0.1403^{fghi}	$0.1023^{ghijklm}$	$0.03867^{pqrstuvwx}$	$0.03233^{rstuvwx}$
M19	0.1543^{efg}	0.1457^{fgh}	0.02767^{tuvwx}	0^{x}
M20	$0.08433^{jklmnopqr}$	$0.08033^{klmnopqrs}$	0.02500^{tuvwx}	0^{x}

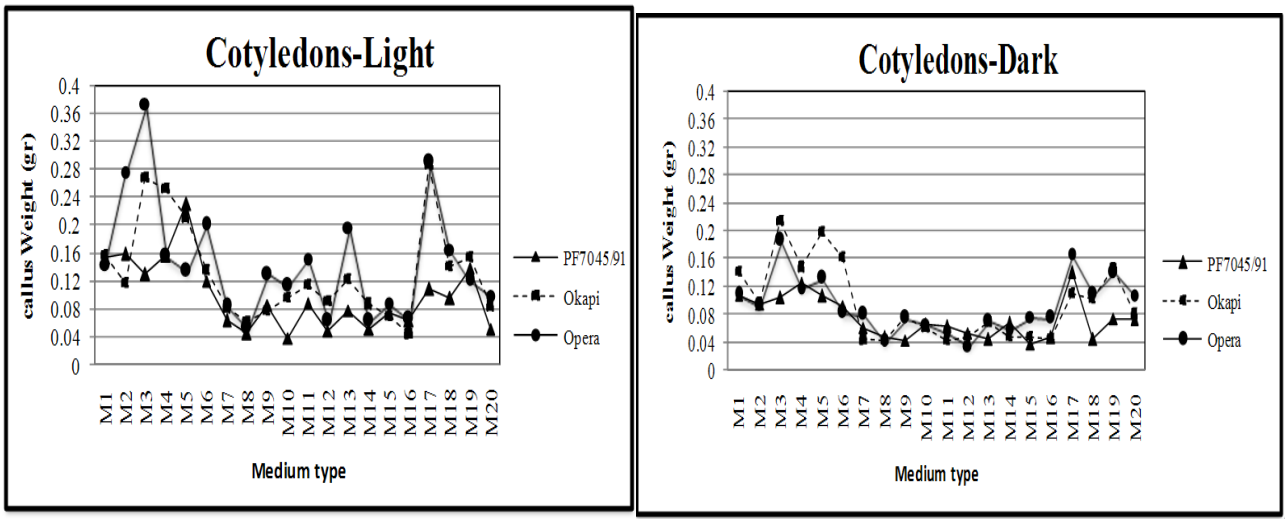

Fig 4. Weight of cotyledon-derived calli in three rapeseed cultivars *Opera*, *Okapi* and *PF7045/91*, grown in the light and dark

which have the capacity to form organs and somatic embryos (Chawla 2002; Sane et al., 2006).

Light

Light had a significant effect on increasing calli fresh weight both in cotyledon (Fig. 3-A and 4) and hypocotyl (Fig. 3-B and 5) derived calli (Table 3, 4 and 5). This was also reported in some other plant species (George et al., 2008). When it comes to callus induction, light had a positive effect on callus induction in hypocotyl derived calli since callus induction had increased significantly in the light. Although the fresh weight of cotyledon derived calli have increased in the light, but browning and necrosis of calli increased. The reason for this phenomenon is the poly-phenolic compounds present in the plants. When the explants are cut, these compounds will be oxidized by the polyphenoloxidase enzymes and this is one of the main reasons of browning in the plants calli (Chawla 2002). It is likely that the products of this oxidation process are formed in the light (Chawla 2002), this can be the reason why browning occurs less in the dark. Thus, although the fresh weight of cotyledon derived calli increased in the light, it would be more beneficial to culture these calli in the dark. Other alternatives are addition of antioxidants (like ascorbic acid and citric acid) to the media, soaking in these antioxidants before culturing in the media or subculture to a fresh media immediately after browning has been observed (Chawla 2002). During this experiment no browning was

Table 5. Effects of explants, photoenvironment and different media on calli fresh weight (gram) in cultivar *Opera*; values represent the mean of three replicates. Means followed by the same letter are not significantly different (Tukey's Honestly Significant Difference Test, Probability level= 0.05). Bold numbers show the highest mean in each explant and photoenvironment condition, the underlined numbers show the best conditions for calli induction and growth in each explant and photoenvironment condition.

Opera	Cotyledon-Light	Cotyledon-Dark	Hypocotyl-Light	Hypocotyl-Dark
M1	0.1440^{defgh}	0.1093^{ghijklmnop}	0.03467$^{vwxyz[\]}$	0.01733$^{z[\]}$
M2	0.2767^{b}	0.09300^{hijklmnopqrs}	0.05067$^{rstuvwxyz[\]}$	0.04567$^{stuvwxyz[\]}$
M3	0.3747^{a}	0.1877^{cd}	0.1057^{ghijklmnopq}	0.06333$^{opqrstuvwxyz[}$
M4	0.1557^{cdefg}	0.1187^{efghijklm}	0.07267^{lmnopqrstuvwxy}	0.04967$^{rstuvwxyz[\]}$
M5	0.1353^{efghi}	0.1323^{efghijk}	0.08033^{jklmnopqrstuvw}	0$^{]}$
M6	0.2000^{c}	0.08367^{ijklmnopqrstuv}	0.08733^{ijklmnopqrstu}	0$^{]}$
M7	0.08467^{ijklmnopqrstuv}	0.08100^{jklmnopqrstuv}	0.04567$^{stuvwxyz[\]}$	0$^{]}$
M8	0.05200$^{rstuvwxyz[\]}$	0.04100$^{stuvwxyz[\]}$	0.007667$^{\]}$	0$^{]}$
M9	0.1300^{efghij}	0.07533^{lmnopqrstuvwx}	0.02833$^{wxyz[\]}$	0.02200$^{yz[\]}$
M10	0.1137^{fghijklmno}	0.06667^{mnopqrstuvwxyz}	0.03300$^{vwxyz[\]}$	0.02233$^{yz[\]}$
M11	0.1520^{cdefg}	0.05400$^{qrstuvwxyz[\}$	0.01433$^{[\]}$	0.01533$^{z[\]}$
M12	0.06333$^{opqrstuvwxyz[}$	0.03533$^{uvwxyz[\]}$	0.01333$^{[\]}$	0$^{]}$
M13	0.1973^{c}	0.07167^{lmnopqrstuvwxy}	0.02200$^{yz[\]}$	0$^{]}$
M14	0.06300$^{opqrstuvwxyz[}$	0.05867$^{pqrstuvwxyz[\}$	0.02167$^{yz[\]}$	0$^{]}$
M15	0.08800^{ijklmnopqrst}	0.07767^{klmnopqrstuvwx}	0$^{]}$	0$^{]}$
M16	0.06533$^{nopqrstuvwxyz[}$	0.07533^{lmnopqrstuvwx}	0$^{]}$	0.01367$^{[\]}$
M17	0.2903^{b}	0.1687^{cde}	0.1157^{fghijklmn}	0.02700$^{xyz[\]}$
M18	0.1653^{cdef}	0.1107^{ghijklmnop}	0.04967$^{rstuvwxyz[\]}$	0.03267$^{vwxyz[\]}$
M19	0.1210^{efghijkl}	0.1413^{defgh}	0.04067$^{tuvwxyz[\]}$	0$^{]}$
M20	0.09900^{hijklmnopqr}	0.1080^{ghijklmnop}	0$^{]}$	0$^{]}$

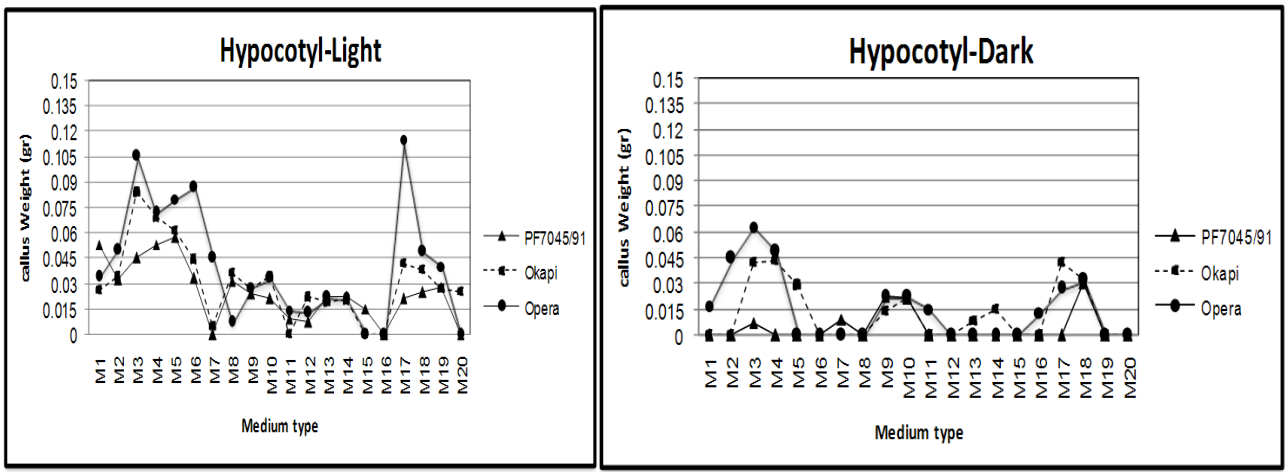

Fig 5. Weight of hypocotyl-derived calli in three rapeseed cultivars *Opera*, *Okapi* and *PF7045/91*, grown in the light and dark.

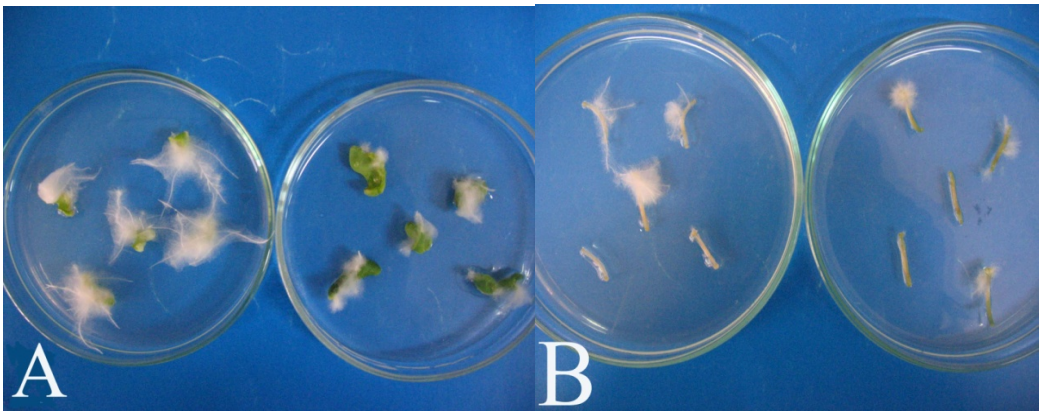

Fig 6. Root formation and growth in the dark and light. (A) Roots formed on *Okapi* cotyledons which were in media M13, the left Petri dish was put in the dark and the right one in the light. (B) Roots formed on *Okapi* hypocotyls which were in media M10, the left Petri dish was put in the dark and the right one in the light.

observed in hypocotyl derived calli, even in the light. This may show that hypocotyl sections contain less poly-phenolic compounds. Light seems to have effect on root growth. Although no difference in root formation were observed in light and dark, however, root growth enhanced in the dark (Fig. 6). The reason for this enhancement may lay in the fact that natural auxin levels increases in the darkness and CKs synthesis stimulated to occur in red light (George et al., 2008). In summary, in the present study we introduced better explants, PGRs and environmental combinations for higher and better rapeseed callus induction and growth. It seems under various conditions, the responses of cultivars were different. Light is an important factor and the responses of cultivars in various media could be different under this condition.

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