http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (16): 2040-2043, 2008
ISSN 1028-8880

Effects of Genotype and $AgNO_3$ on Shoot Regeneration in Winter Cultivars of Rapeseed (*Brassica napus*)

[1]E.D. Uliaie, [1]M. Farsi, [2]B. Ghreyazie and [3]J. Imani
[1]Department of Biotechnology and Plant Breeding, College of Agriculture,
Ferdowsi University of Mashhad, Mashhad, Iran
[2]Department of Genomics, Agricultural Biotechnology Research Institute of Iran,
Seed and Plant Improvement Institutes Campus, P.O. Box 31535-1897, Mahdasht Road, Karaj, Iran
[3]Justus-Liebig University, Institute of Phytopathology and Applied Zoology,
Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany

Abstract: Ability of shoot regeneration from cotyledon explants of rapeseed (*Brassica napus*) winter genotypes and the effect of silver nitrate and naphthalene acetic acid in shoot regeneration were investigated. The optimum medium for regeneration was the medium supplemented with 3 mg L^{-1} 6-Benzylaminopurine and 0.15 mg L^{-1} 1-naphthaleneacetic acid. The addition of 5 mg L^{-1} silver nitrate significantly improved shoot regeneration. Shoot regeneration response was strongly different between genotypes with a range of variation from 79% in spring genotype PF and 7% in Okpi. The highest root production was recorded on medium containing indol-3-butyric acid. The rooted plants successfully transferred to soil and adapted to greenhouse conditions. No abnormally was observed and the regenerated plants were morphologically similar to the field grown parental plants.

Key words: Tissue culture, regeneration, rooting, silver nitrate

INTRODUCTION

Canola (*Brassica napus* L.) is an important oil crop, ranking third after soybean and palm oil in global production. Of all the edible vegetable oils widely available today, canola has the lowest saturated fat content, making it appealing to health-conscious consumers (Cardoza and Stewart, 2003).

Genetic engineering offers an additional possibility of introducing new traits into a wide range of existing commercial cultivars. *In vitro* regeneration of whole fertile plants from appropriate tissues is important in genetic plant transformation.

From an agronomical and an environmental point of view, initiatives in growing winter cultivars of *B. napus* have increased during recent years due to the higher yield of winter crops and due to the capability of winter crops to utilize excess nitrogen fertilizer in the fields during the winter period (Damgaard *et al*., 1997). Regeneration in *B. napus* is highly variable and genotype specific (Khan *et al*., 2002). Therefore, the majority of transformation studies on *B. napus* has been performed with spring cultivars and the genotypes have largely been restricted to a few cultivars, with the cultivar Westar the predominant genotype. Previous studies describing transformation and regeneration protocols for rapeseed have emphasized the limitations in the regeneration potential of rapeseed winter cultivars (Damgaard *et al*., 1997).

Ethylene is produced by plants and is known to have various effects on plant tissue cultures. In recent years there has been increasing evidence that the occurrence of morphogenesis in cultured plant cells may be associated with ethylene. The influence of ethylene inhibitors such as silver nitrate in *in vitro* culture of plants have been reported by Curtis *et al*. (2004), Akasaka-Kennedy *et al*. (2005), Ozden-Tokatli *et al*. (2005) and Mundhara and Rashid (2006).

The aim of the present study is to establish an efficient protocol for shoot regeneration in some winter varieties of *B. napus* and evaluate the effect of silver nitrate on shoot regeneration from cotyledon explants.

MATERIALS AND METHODS

The experiments were performed at the Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad during 2007. The seeds of all varieties were kindly provided by Agriculture and Natural Resources Research Center of Razavi Khorasan,

Corresponding Author: Ebrahim Dorani Uliaie, Department of Biotechnology and Plant Breeding, College of Agriculture, Ferdowsi University of Mashhad, P.O. Box 9177948978, Mashhad, Iran
Tel: (+98-511)-8796818-20 Fax: (+98-511)-8787430

Mashhad, Iran. Induction of shoot regeneration from cotyledonary explants was carried out according to Moloney *et al.* (1989) with minor modifications. Seeds were surface sterilized with 70% ethanol for 1 min and 5% hypochlorite sodium for 15 min and subsequently rinsed 3 times with sterilized water. Sterilized seeds were germinated on solid basal 1/2 MS medium supplemented with 2% sucrose. The pH of medium was adjusted to 5.7 and autoclaved at 121°C for 20 min. Silver nitrate was filter-sterilized and added to the autoclaved media. The germination condition were 25°C, 8: 16 h photoperiod at 40 μmol m^{-2} sec. Cotyledons, including the 1-2 mm petiole were excised from 5-day-old seedlings and cultured on regeneration medium at 15 cotyledons per 100×20 mm plate. All plates were sealed with gas permeable tape and maintained at 25±2°C under 16 h photoperiod and irradiance of 40-50 μmol m^{-2} sec. The experiment was carried out in a factorial experiment with randomized block design layout. The treatments were two concentrations of 6-Benzylaminopurine (BAP) (1.5 and 3 mg L^{-1}), two concentration of naphthaleneacetic acid (NAA) (0 and 1.5 mg L^{-1}) and two concentration of $AgNO_3$ (0 and 5 mg L^{-1}). Treatments were replicated three times and every plate consisted 15 explants. The number of shoot induced explants was counted in each plate. Shoots induced in cotyledon explants were transferred to 1/2 MS agar medium containing various auxins or lacking plant growth regulators for development of root system. Regenerated plants were grown in vermiculite for about 2 weeks before transfer to soil in a greenhouse.

Analysis of variance were carried out using of MSTATC software and significant differences among the means were assessed by Duncan's test on each experiment, at 1% probability level.

RESULTS AND DISCUSSION

Callus formation from cut end of cotyledons from all the cultivars except Okpi was observed within one week. The explants of Okpi became necrotic at the beginning of the experiment. The important role of BAP for shoot differentiation in *Brassica* cotyledons has been reported previously (Moloney *et al.*, 1989). Hachey *et al.* (1991) and Guo *et al.* (2005) described a requirement of NAA for the shoot regeneration from *B. campestris* and *B. juncea* cotyledons, respectively. Using the NAA in low concentration (0.15 mg L^{-1}) significantly increased shoot regeneration in all cultivars, which is in agreement with results on *B. campestris* and *B. juncea* (Hachey *et al.*, 1991; Guo *et al.*, 2005). In the presence of NAA in the medium, shoot regeneration exhibited a delay phase in comparison with that in an NAA free media due to brief callus induction period. The presence low concentration of NAA in parallel with BAP caused the concurrent formation of roots along with shoots from the explant. The higher concentration of BAP gave better results in regeneration and the maximum shoot regeneration frequency was obtained in medium supplemented with 3 mg L^{-1} BA and 0.15 mg L^{-1} NAA.

$AgNO_3$ is another important factor in affecting the rate of shoot regeneration. A high frequency of shoot regeneration in Orient and SLM cultivars was obtained only when $AgNO_3$ was included in culture medium (Fig. 1). This response is in contrast to the findings of Hachey *et al.* (1991) where it reduced shoot regeneration *Brassica campestris* using cotyledon explants. The effect of $AgNO_3$ in stimulation of shoot morphogenesis have been reported by Brar *et al.* (1999), Zhang *et al.* (2001), Curtis *et al.* (2004), Akasaka-Kennedy *et al.* (2005), Ozden-Tokatli *et al.* (2005) and Mundhara and Rashid (2006). Regeneration percentage in cultivars. PF, Cobra and Okpi was least modified by the addition of silver nitrate. Although, 5 mg L^{-1} $AgNO_3$ slightly increased the regeneration percentage in those cultivars, but the effect was insignificant (Fig. 1). This genotype-dependent effect of $AgNO_3$ in *in vitro* culture have been reported in *Phoenix dactylifera* (Williams *et al.*, 1990) and *Brassica oleracea* (Al-Khayri and Al-Bahrany, 2004).

Silver nitrate is a well known inhibitor of ethylene action (Beyer, 1976) and since ethylene inhibits auxin transport, one of its functions may be to allow polar auxin transport to the petiole base where regeneration occurs. De Block *et al.* (1989) also reported that $AgNO_3$ was absolutely required for shoot recovery from *B. napus* hypocotyls. It was shown that the use of 3 mg L^{-1} $AgNO_3$ increased the ability of shoot regeneration from *B. napus* leaf explants where in some genotype there was no regeneration in absence of $AgNO_3$ (Akasaka-Kennedy *et al.*, 2005).

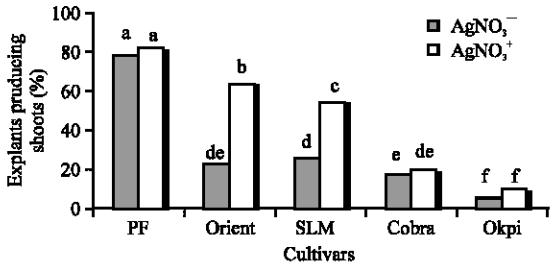

Fig. 1: Effect of addition of $AgNO_3$ (5 mg L^{-1}) on frequency of shoot regeneration on five rapeseed genotypes after 4 weeks. Hatched and open bars indicate no addition of $AgNO_3$ and addition of $AgNO_3$, respectively. Treatments with the same letters are not significantly different (α = 1%)

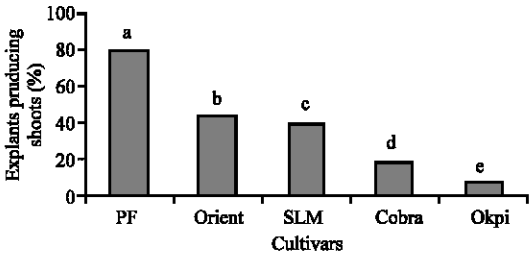

Fig. 2: Effect of genotype on shoot regeneration on five days old cotyledon explant. Treatments with the different letters are significantly different (α = 1%)

Considerable variation in shoot regeneration from cotyledon explants was observed within used genotype. The Okapi and Cobra were the more recalcitrant genotype and PF was relatively easy in terms of regeneration with the high shoot regeneration frequency. The frequency of shoot regeneration ranged between 79% in PF, 18 and 7% in Cobra and Okpi, respectively (Fig. 2). Similar conclusions was also drawn for *Brassica campestris* (Hachey *et al*., 1991), *Brassica napus* where using the leaf explants (Akasaka-Kennedy *et al*., 2005) *Capsicum annuum* (Balazs *et al*., 2008). The results indicate that shoot regeneration ability is strongly influenced by the genotype used.

Rooting of regenerated shoots is one of the problems encountered in canola transformation studies. The rooting of *in vitro* shoots, using half-strength medium has, been reported by Samantaray *et al*. (1995) and Upreti and Dhar (1996). Decreasing the sucrose concentration in the rooting medium has proven to be beneficial for root induction (Kooi *et al*., 1999; Figueiredo *et al*., 2001). In our study, we used half-strength MS medium supplemented with a lower concentration of sucrose (10 g L^{-1}) and three different auxins. Although root formation was observed in growth regulator-free medium, the presence of auxin was effective in increasing root formation (Fig. 3). The highest root production was recorded in a medium containing 2 mg L^{-1} IBA, where almost all shoots, formed roots within 2 weeks. However the medium containing 2 mg L^{-1} NAA, were also effective for root formation, but the root system was qualitatively poorer than roots growing in 2 mg L^{-1} IBA medium, especially for the root length.

Five of the rooted shoots were transplanted to vermiculite. After one week, they were transferred to soil and kept in greenhouse for further growth. The plants were not morphologically different from the parental plants; as they produced normal flowers and set abundant seeds.

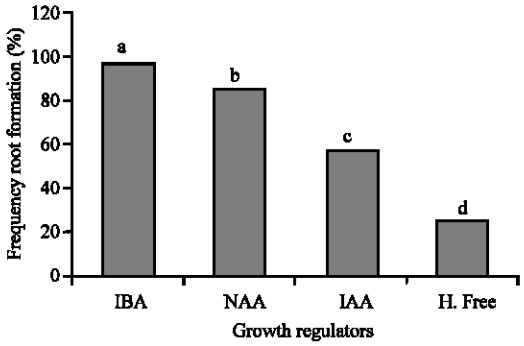

Fig. 3: Effect of various growth regulators on root formation on regenerated shoot from cotyledon explant of *B. napus* cv. Orient. Data collected from 4 replicates and then regenerated shoots were used as one replicate. Same letters on the bar are not significantly different at the 1% level

These results demonstrated the positive effect of NAA and genotype-dependent stimulatory effect of $AgNO_3$ on optimum regeneration from cotyledons in *B. napus*. Genotypes that showed a high level of shoot formation may be useful for genetic engineering. We are currently subjecting the PF and Orient genotypes to genetic transformation for salt tolerance.

ACKNOWLEDGMENT

We thank Agriculture and Natural Resources Research Center of Razavi Khorasan for kindly providing the seeds used in this study.

REFERENCES

Akasaka-Kennedy, Y., H. Yoshida and Y. Takahata, 2005. Efficient plant regeneration from leaves of rapeseed (*Brassica napus* L.). The influence of $AgNO_3$ and genotype. Plant Cell Rep., 24: 649-654.

Al-Khayri, J.M. and A.M. Al-Bahrany, 2004. Genotype-dependent *in vitro* response of date palm (*Phoenix dactylifera* L.) cultivars to silver nitrate. Scin. Hortic., 99: 153-162.

Balazs, E.A., M. Csanyri, G. Csillery, Z. Diveki and I. Nagy *et al*., 2008. Evaluation of a wide range of pepper genotypes for regeneration and transformation with an *Agrobacterium tumefaciens* shooter strain. S. Afr. J. Bot., 10.1016/j.sajb.2008. 05.005.

Beyer, E.M., 1976. A potent inhibitor of ethylene action in plants. Plant Physiol., 58: 268-271.

Brar, M.S., M.J. Moore, J.M. Al-Khayri, T.E. Morelock and E.J. Anderson, 1999. Ethylene inhibitors promote *in vitro* regeneration of cowpea (*Vigna unguiculata* L.). *In vitro* Cell. Dev. Biol. Plant, 35: 222-225.

Cardoza, V. and C.N. Stewart, 2003. Increased *Agrobacterium*-mediated transformation and rooting efficiencies in canola (*Brassica napus* L.) from hypocotyl segment explants. Plant Cell Rep., 21: 599-604.

Curtis, I.S., H.G. Nam and K. Sakamoto, 2004. Optimized shoot regeneration system for the commercial Korean Radish Jin Ju Dae Pyong. Plant Cell Tissue Organ Cult., 77: 81-87.

Damgaard, O., L.H. Jensen and O.S. Rasmussen, 1997. *Agrobacterium tumefaciens* mediated transformation of *Brassica napus* winter cultivars. Transgenic Res., 6: 279-288.

De Block, M., D. Debrouwer and P. Tenning, 1989. Transformation of *Brassica napus* and *Brassica oleracea* using *Agrobacterium tumefaciens* and the expression of the *bar* and *neo* genes in the transgenic plants. Plant Physiol., 91: 694-701.

Figueiredo, S., A. Norma and V.R.C. Viana, 2001. Micropropagation of *Rolliniamucosa* (Jacq). Baill. *In vitro* Cell Dev. Biol. Plant., 37: 471-475.

Guo, D.P., Z.J. Zhu, X.X. Hu and S.J. Zheng, 2005. Effect of cytokinins on shoot regeneration from cotyledon and leaf segment of stem mustard (*Brassica juncea* var. *tsatsai*). Plant Cell Tissue Organ Cult., 83: 123-127.

Hachey J.E., K.K. Sharma and M.M. Moloney, 1991. Efficient shoot regeneration of *Brassica campestris* using cotyledon explants cultured *in vitro*. Plant Cell Rep., 9: 549-554.

Khan, M.R., H. Rashid and A. Quraishi, 2002. High frequency shoot regeneration from Hypocotyl of Canola (*Brassica napus* L.) cv. Dunkled. Plant Tissue Cult., 75: 131-138.

Kooi L.T., C.L. Keng and C.T.K. Hoe, 1999. *In vitro* rooting of sentang shoots (*Azadirachta excelsa* L.) and acclimatization of the plantlets. *In vitro* Cell Dev. Biol. Plant, 35: 396-400.

Moloney, M.M., J.M. Walker and K.K. Sharma, 1989. High efficiency transformation of *Brassica napus* using *Agrobacterium* vectors. Plant Cell Rep., 8: 238-238.

Mundhara, R. and A. Rashid, 2006. TDZ-induced triple-response and shoot formation on intact seedlings of Linum, putative role of ethylene in regeneration. Plant Sci., 170: 185-190.

Ozden-Tokatli, Y., E.A. Ozudogru and A. Akcin, 2005. *In vitro* response of pistachio nodal explants to silver nitrate. Sci. Hortic., 106: 415-426.

Samantaray S.G., R. Rout and P. Das, 1995. An *in vitro* study on organogenesis in *Trema orientalis* (Blume) Linn. Plant Sci., 105: 87-94.

Upreti, J. and U. Dhar, 1996. Micropropagation of *Bauhinia vahlii* Wight and Arnott. A leguminous liana. Plant Cell Rep., 16: 250-254.

Williams, J.D., A.C. Pink and N.L. Biddington, 1990. Effect of silver nitrate on long-term culture and regeneration of callus from *Brassica oleracea* var. *gemmifera*. Plant Cell Tissue Organ Cult., 21: 61-66.

Zhang, P., S. Phansiri and J. Puonti-Kaerlas, 2001. Improvement of cassava shoot organogenesis by the use of silver nitrate *in vitro*. Plant Cell Tissue Organ Cult., 67: 47-54.