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MICROPROPAGATION OF LENTIL (*LENS CULINARIS* MEDIK.) USING PULSE TREATMENT OF IMMATURE PLUMULAR APICES

Muhammad Aasim*

Department of Biology, Kamil Ozdag Faculty of Science,
Karamanoglu Mehmetbey University Karaman, Ankara, Turkey
***Corresponding author's e-mail: mshazim@gmail.com**

Lentil is highly recalcitrant and is difficult to regenerate through tissue culture. The study is aimed to overcome this problem by developing an efficient regeneration system using immature plumular apice explants from immature zygotic embryos of Turkish lentil cv. Ciftci. The results showed that 10 mg/l BA pulse treatment of explants for 10 days followed by culture on MS medium containing various concentrations of BA-IBA supplemented with activated charcoal and PVP affected shoot regeneration frequency, mean number of shoots per explant and shoot length. Irrespective of the pulse treatment, combination of BA with IBA in MS medium promoted longer shoots compared to any concentration of BA alone. Maximum number of shoots (4.25) per explant was recorded on MS medium containing 0.25 mg/l BA + 0.1 mg/l IBA after pulse treatment. The longest shoots (6.17 cm) were recorded in pulse treated explants when cultured on MS medium containing 0.25 mg/l BA + 0.1 mg/l IBA. The regenerated shoots were rooted on MS medium containing 0.25 to 1 mg/l IBA or 1 mg/l IAA. The rooted plants were acclimatized at 24±2^{o}C in the growth room where, they flowered and set seeds.

Keywords: *in vitro*, plumular apices, explant, legume, lentil, shoot regeneration, growth regulators.

Abbreviations: BA-6Benzyladenine, IAA-Indole 3 acetic acid, IBA-Indole 3 butyric acid, PVP-Polyvinylpyrrolidone

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important seed legume crop. It is one of the oldest grain legumes domesticated (Bahl *et al.,* 1993). Its contribution to human nourishment is of vital importance in many areas of the world. It provides one of the best means of overcoming malnutrition among people in developing countries (Savage, 1988; Zahran 1999). Besides traditional areas of cultivation like Turkey and South Asia, it is also cultivated in subtropical and northern hemisphere such as Canada and Pacific Northwest regions. Its seeds provide an excellent source of carbohydrates, dietary fibers, and balanced range of minerals, protein and vitamins (Christou, 1994; Adsule *et al.,* 1989). Lentil can fix atmospheric nitrogen at the maximum daily rate of 4.4 kg ha^{-1} day^{-l} (Van Kessel, 1994). This ability to fix atmospheric nitrogen helps in growth of lentil itself and also the crops that are grown thereafter (Zahran, 1999). Successful regeneration of legumes has been achieved by species specific determination of critical regeneration parameters such as explant source, genotype, media constituents and temperature (Khawar and Ozcan, 2002a). Lentils are considered recalcitrant to cell and tissue culture and most difficult to regenerate whole plants due to difficulties in root induction (Fratini and Ruiz, 2002; Fratini *et al.,* 2003). Shoot regeneration of lentil has been previously reported from meristem tips, shoot tips and shoot meristems (Bajaj and Dhanju, 1979; Williams and McHughen, 1986; Singh and Raghuvanshi, 1989; Polanco and Ruiz, 1997), epicotyl (Williams and McHughen, 1986), embryonic axes (Saxena and King, 1987), first nodes and bractlets of immature seeds (Polanco *et al.,* 1988), cotyledonary seedlings (Mallick and Rashid, 1989), nodal segments (Singh and Raghuvanshi, 1989), stem and cotyledonary nodes (Warkentin and McHughen, 1993; Polanco, 2001; Khawar and Ozcan 2002a, Sarker *et al.,* 2003; Khawar *et al.,* 2004; Sevimay *et al.,* 2005) and decapitated embryo (Sarker *et al.,* 2003). However, all of the described procedures generally have yielded insufficient regeneration frequency. Moreover, these have involved extensive manipulation of culture conditions or faced other problems during regeneration. Hence, the development of more reliable micropropagation system in lentil is essential to complement conventional breeding programs in lentil.

Although, shoot regeneration protocols from plumule explant in legumes like pea (Molnar *et al.,* 1999), pigeon pea (Surekha *et al*., 2005) and cowpea (Aasim *et al.,* 2009) have been reported previously; no previous reports on shoot regeneration from immature plumular apice obtained from immature seeds of lentil exist so far. Ethylene, a naturally occurring gaseous plant hormone is responsible for inducing many biochemical processes leading to programmed cell death. It also activates senescence related gene transcription (Woodson and Lawton, 1988; Lawton *et al.,* 1990) and induction of recalcitrants in lentils. Cytokinins have been known as growth regulators, which markedly reverse senescence in various plant species (Skutnik *et al.,* 1999). These help to maintain the cells younger and improve their regeneration. Application of cytokinins reduces water stress

damage, respiration rates and sensitivity to ethylene. Furthermore, cytokinins improve water uptake, and inhibit ethylene production to (Goszczynska *et al.,* 1985). Therefore, this, study is aimed at exploring the *in vitro* regeneration potential of BA pulse-treated plumule apice explants of highly recalcitrant lentil plants. Efficient methods for inducing shoot regeneration in lentils can help to develop genetically modified lentil through *Agrobacterium* mediated genetic transformation in future.

MATERIALS AND METHODS

Lentil pods with green seeds were collected two week before harvest from the experimental fields at the Department of Field Crops, Ankara University, Ankara, Turkey. The pods were surface sterilized with 100% commercial bleach (Ace, Turkey, containing 5% NaOCl) for 10 min by continuous stirring using a digital magnetic stirrer (MS-Pro - China) followed by 3×3 min rinsing with sterilized distilled water. The immature seeds from the sterilized pods were cut opened to obtain immature embryos. These were separated into two sets. One set was pulse treated for 10 days on agar solidified MS medium (Murashige and Skoog, 1962) containing 10 mg/l BA (Duchefa, The Netherlands) and the the other set was cultured on MS medium devoid of BA and pulse treatment (control). Thereafter, plumular apices were obtained from both sets after 10 day of culture and explants were cultured on MS medium supplemented with 3% sucrose, 4 g/l activated charcoal (Sigma St. Lo MO), 1.0% PVP (Polyvinylpyrrolidone), and 0.65% agar containing 0.25, 0.50, 0.75 and 1 mg/l BA, together with 0 and 0.1 mg/l IBA. The experiment was run in triplicate with the pH of all media adjusted to 5.8 before autoclaving (118 kPa atmospheric pressure, 120^{o}C for 21 min). All cultures were incubated under 16h photoperiod (4000 lux) provided by Philips® cool white fluorescent tubes. The explants were subcultured at 4 weeks interval. The final data were recorded after 8 weeks. The regenerated shoots were rooted on agar-solidified MS rooting medium containing 0.25, 0.50 and 1.0 mg/l IBA or IAA alone or 0.25, 0.50 and 1.0 mg/l IBA with 0.25, 0.50 and 1.0 mg/l IAA in Magenta GA7 vessels. After 16 week, *in vitro* grown rooted plants were removed from the adhering gel, transplanted to earthen pots containing sand, clay and organic matter soil mix (1:1:1). They were acclimatized in growth rooms under 80% humidity during first 7 days followed by gradual reduction to 40% humidity in 10 days' time at 26-32^{o}C. Each treatment contained 4 explants and was replicated 6 times (4 x 6 = 24 explants) in both shoot and root regeneration experiments. All experiments were repeated twice (4 x 6 x 2 = 48 explants). Statistical analysis was performed as One Way ANOVA using SPSS17 for Windows and post hoc tests were performed using LSD or t-test. Data given in percentages were subjected to arcsine transformation (Snedecor and Cocharan, 1967) before statistical analysis.

RESULTS AND DISCUSSION

The study describes potential of whole plant regeneration from previously unreported immature plumular apices of immature lentil seeds on MS medium containing BA with and without IBA followed by rooting and acclimatization. The results also emphasized significant role of the pulse treatment to axillary shoot regeneration.

***Bud regeneration from immature plumular apices on MS medium containing BA*:** No bud initiation or shoot regeneration was recorded on pulsed or non-pulsed explants cultured on MS medium with activated charcoal and PVP (Table 1). However, pulse treated explants induced variable number of shoot buds, but did not convert into shoots (data not tabulated and presented) (control, Table 1). The shoot regeneration data was taken after eight weeks of culture. Both pulsed and non-pulsed immature plumular apices initiated multiple axillary shoot buds on various concentrations of BA with activated charcoal or PVP within 7-11 days of culture. Pulsed explants indicated a consistent increase in shoot length on MS medium containing various concentrations of BA with 0.1 mg/l IBA. It also showed 100% shoot regeneration with mean number of 2.25 to 3.78 shoots per explant and 2.09 to 3.58 cm long shoots which is

Table 1. Effect of various concentrations of BA on shoot regeneration behavior of plumular apices explants of Turkish Lentil (*L. culinaris* Medik.) cv. Ciftci

BA (mg/1)	Frequency of shoot regeneration (%)		Mean number of shoots per explant		Shoot length (cm)	
	Pulsed explants	Non-pulsed explants	Pulsed explants	Non-pulsed explants	Pulsed explants	Non-pulsed explants
0.25	100.00	58.33ab	3.78a	1.33a	2.09b	0.14b
0.50	100.00	16.67b	2.50bc	0.53b	3.02ab	1.11ab
0.75	100.00	58.33ab	3.08a	1.01ab	3.33ab	1.15ab
1.00	100.00	66.67a	2.25c	1.21a	3.58a	1.15ab
Control (MS medium)	0.00	0.00	0.00	0.00	0.00	0.00

Means followed by different small letters within columns are sigificantly different using LSD test at P<0.005

in line with the findings of Aasim *et al.* (2008, 2009, 2010). They also suggested that if the culture media contained BA in the presence of auxins, it promotes shoot length of cowpea. Contrarily, Aasim *et al.* (2011) reported negative effects of auxins in combination with BA on shoot length of chickpea. Non-pulsed explants (Table 1) induced 16.67 to 66.67% shoots, 0.53 to 1.33 shoots per explant with shoot length of 0.14 to 1.15 cm on MS medium containing various concentrations of BA and these findings are in agreement with Bajaj and Dhanju (1979), Singh and Raghuvanshi (1989), Saxena and King (1987), Polanco *et al.* (1988), Mallick and Rashid (1989), Malik and Saxena (1992), Warkentin and McHughen (1993), Polanco and Ruiz (1997, 2001), Fratini and Ruiz (2002), Fratini *et al.* (2003), Khawar and Ozcan (2002a), Sarker *et al.* (2003), Khawar *et al.* (2004) and Sevimay *et al.* (2005). These authors also suggested variable shoot regeneration behavior of different explants of lentil on different concentrations and combinations of plant growth regulators.

Bud regeneration from immature plumular apices on MS medium containing BA and 0.1mg/l IBA: Pulsed or non-pulsed explants cultured on MS medium with activated charcoal and PVP (Table 2) failed to induce bud initiation or shoot regeneration (control, Table 2). Contrarily, pulse treated explants induced shoot buds that did not convert into shoots (data not tabulated and presented). The shoot regeneration data was taken after eight weeks of culture showing positive effect of pulse treatment on all attributes of shoot regeneration with no signs of inhibition (Table 2). The explants developed multiple shoot buds on MS medium containing different levels of BA and 0.1 mg/l IBA after 9-12 days of culture. Number of shoots per explant ranged 2.17 to 4.25 with shoot length range of 2.33 to 6.17 cm. Maximum number of shoots per pulsed explant was recorded on MS medium containing 0.25 mg/l BA and 0.1 mg/l IBA. The number of shoots per explant on all other concentrations of BA and 0.1 mg/l IBA reduced dramatically but with longer shoots reaching up to 6.17 cm on MS medium containing 0.5 mg/l BA and 0.1 mg/l IBA. On the contrary, variable shoot regeneration behavior was noted on non-pulsed immature plumular apices cultured on MS medium containing various levels of BA and 0.1 mg/l IBA. Non-pulsed explants were slow in axillary bud regeneration that could only be detected after 15-20 days of culture. They showed sharply reduced axillary shoot development on MS medium containing 0.50-1 mg/l BA with 0.1 mg/l IBA. The results showed that 0.5-1 mg/l BA and 0.1 mg/l IBA influenced shoot regeneration negatively. Shoot regeneration ranged 8.33 to 83.33% with 0.11 to 2.17 shoots per explant and shoot length of 0.51 to 1.58 cm. Among variants of BA and BA-0.1 mg/l IBA used in this study, MS medium containing 0.25 mg/l BA and 0.1 mg/l IBA induced maximum number of axillary shoots per pulsed explant. The results support findings of Aasim *et al.* (2008, 2009); who had similar observations in shoot regeneration from shoot meristems and pulse treated-plumular apice of cowpea.

***Shoot length from immature plumular apices on MS medium containing variants of BA and BA-0.1mg/l IBA*:** Positive increase in the shoot length was recorded on MS regeneration medium containing different concentrations of BA-IBA compared to various levels of BA used alone. The results are in line with the findings of Aasim *et al.* (2008, 2009, 2010). They also suggested that if the culture media contain BA in the presence of auxins, it promotes shoot length of cowpea. Contrarily, Aasim *et al.* (2011) reported negative effect of auxins in combination with BA on shoot length of chickpea.

***Root induction and acclimatization*:** The study on induction of adventitious roots in lentil is highly important from practical point, since previous studies suggests difficulty in rooting of lentil microcuttings (Bajaj and Dhanju, 1979; Singh and Raghuvanshi, 1989; Saxena and King, 1987; Polanco *et al.*, 1988; Mallick and Rashid, 1989; Malik and Saxena, 1992; Warkentin and McHughen, 1993; Polanco and Ruiz, 1997, 2001; Fratini and Ruiz, 2002; Fratini *et al.*, 2003; Khawar and Ozcan, 2002a; Sarker *et al.*, 2003; Khawar *et al.*, 2004; Sevimay *et al.*, 2005).

Table 2. Effect of various concentrations of BA-IBA on shoot regeneration behavior of plumular apices explants of Turkish Lentil (*L. culinaris* Medik.) cv. Ciftci

BA (mg/1)	IBA (mg/1)	Frequency of shoot regeneration (%)		Mean number of shoots per explant		Shoot length (cm)	
		Pulsed explants	Non-pulsed explants	Pulsed explants	Non-pulsed explants	Pulsed explants	Non-pulsed explants
0.25	0.1	100.00	83.33a	4.25a	2.17a	2.33c	0.51c
0.50	0.1	100.00	8.33b	2.50b	0.32b	6.17a	1.58a
0.75	0.1	100.00	8.33b	2.17c	0.11b	4.00b	1.23b
1.00	0.1	100.00	8.33b	2.50b	0.17b	3.98b	1.19b
Control (MS medium)		0.00	0.00	0.00	0.00	0.00	0.00

Means followed by different small letters within columns are sigificantly different using LSD test at P<0.005

***Rooting on MS medium containing various concentration of IBA-IAA*:** The explants failed to root on MS medium containing IBA used in combination with IAA (results not presented). This suggests that combinations of any concentration of IBA with any concentration of IAA in the rooting medium is inhibiting. Ludwig-Mtiller *et al.* (1993) found that the two hormones have different modes of conjugation; such that IAA mostly conjugate via amide bonds and the IBA conjugate via ester bonds. Wiesman *et al.* (1988) suggested that the IBA conjugates may be a better source of free auxin than those of IAA. This may explain the higher activity of IBA and its more appropriateness for rooting of lentil compared to IAA. The physiological effect of IBA and IAA on rooting could be speculated in the context of the above studies with different IBA and IAA transport mechanisms; that inhibited rooting when both were present in combination.

***Rooting on MS medium containing various concentration of IAA*:** MS medium containing 0.25 or 0.50 mg/l IAA failed to induce roots. After 9 week of culture, root buds were observed on few shoots on MS medium containing 1 mg/l IAA (Table 3) in partial agreement with Bennet-Clark and Kefford (1953) and Leopold (1955), who noted that indole-3-acetic acid (IAA), which occurs naturally in root apices also inhibits root growth. Ruzicka *et al.* (2007) and Swarup *et al.* (2007) also noted that ethylene stimulates IAA synthesis and transport in root tips, which creates IAA gradients in the root elongation zone, inhibiting cell elongation. However, moderate rooting of 27.78% at 1 mg/l IAA suggested partial creation of a functional polar auxin transport (PAT) system that partially maintained IAA gradients in favor of rooting. Wiesman *et al.* (1988) compared movement and metabolism of indole-3-acetic acid and indole-3-butyric acid in mung bean (*Vigna radiata* L.) cuttings and found that indole-3-butyric acid (IBA) was much more effective than indole-3-acetic acid (IAA) in inducing adventitious root formation.

Table 3. Effect of various concentrations of IAA and IBA on root regeneration behavior of Turkish Lentil (*Lens culinaris* Medik.) cv. Ciftci

Treatments	Frequency of rooting (%)
IAA (mg/l)	
0.25	0.00 b
0.50	0.00 b
1.00	27.78 a
Control (MS medium)	0.00 b
IBA (mg/l)	
0.25	41.67 a
0.50	31.25 b
1.00	12.50 c
Control (MS medium)	0.00 d

Means followed by different letters within columns are sigificantly different using LSD test at P<0.005

***Rooting on MS medium containing various concentration of IBA*:** Root formation started after 16 weeks on rooting medium supplemented with IBA (Table 3). Most of the shoots induced 4-6 secondary shoots along with rhizogenesis arising from the same points on shoots. Rooting frequency ranged 12.50 to 41.67%. Maximum rooting was recorded on MS medium containing 0.25 mg/l IBA. It is suspected that the stem cells of microcuttings in contact with rooting (IBA containing) media may have divided asymmetrically to produce two types of daughter cells; one that retained stem cell identity for initiation of 100% secondary shoots and the others underwent additional divisions and induced roots in a range of 12.5-41.67%. The maintenance of stems cells ensured the continued contribution of new cells for the production of multiple shoots as reported by Xu *et al.* (2006). The results are also in agreement with Khawar and Ozcan (2002b) in lentil, Aasim *et al.* (2008, 2009, 2010) in cowpea and Aasim *et al.* (2011) in chickpea. The phenomenon is not well understood and needs a thorough research for detailed explanation.

Each increase in the concentration of IBA was inhibitory and resulted in sharp decrease in rooting percentage. The results are in agreement with the findings of De Klerk (1999) who pointed out that IBA is the most effective auxin for rhizogenesis. Since IBA is degraded relatively slow by the auxin destroying enzyme systems (Nordstrom *et al.*, 1991) and it moves very slow in the plants, much of it is retained near the site of application (Weaver, 1972). Its slow movement and delayed degradation may be the primary reason for better performance of IBA as compared to IAA. Ahmad *et al.* (2004) is also in agreement with these findings. IBA may also enhance rooting *via* increased internal free IBA or may synergistically modify the action of endogenous synthesis of IAA (Krieken *et al.*, 1993). Contrarily, Abbas *et al.* (2012) obtained better rooting frequency using IAA compared to IBA in endangered plant *Convolvulus scindicus.*

IAA rooted plantlets failed to survive in the growth room and died. About 50% of IBA rooted *in vitro* regenerated plantlets survived and acclimatized easily in the growth room after 25 days of transfer. These were morphologically normal, fertile and set seeds. Successful acclimatization of IBA rooted plantlets compared to IAA rooted plantlets in this study suggests that IBA should be preferred for rooting of lentils micropropagation.

CONCLUSION

Lentil is highly recalcitrant and is difficult to regenerate and root through tissue culture. A system to regenerate lentil through multiple shoots from immature plumular apices is established in cv. Ciftci. It is expected that the protocol will facilitate and help in future breeding and genetic transformation of other lentil cultivars.

REFERENCES

Aasim, M., K.M. Khawar and S. Özcan. 2008. *In vitro* micropropagation from shoot meristems of Turkish cowpea *(Vigna unguiculata* L.) cultivar Akkız. Bangladesh J. Bot. 37:149-154.

Aasim, M., K.M. Khawar and S. Özcan. 2009. *In vitro* micropropagation from plumular apices of Turkish cowpea *(Vigna unguiculata* L.) cultivar Akkiz. Sci. Horti. 122:468-471.

Aasim, M. 2010. *In vitro* shoot regeneration of NAA-pulse treated plumular leaf explants of cowpea. Not. Sci. Biol. 2:60-66.

Aasim, M., S. Day, F. Rezai, M. Hajyzadeh, S.T. Mahmud and S. Ozcan. 2011. *In vitro* shoot regeneration from preconditioned explants of chickpea (*Cicer arietinum L.*) cv. Gokce. Afr. J. Biotech. 10:2020-2023.

Abbas, A., M. Qaisaer and S.W. Khan. 2012. *In vitro* response of *Convolvulus scindicus* to different growth hormones - an attempt to conserve an endangered species. Pak. J. Agri. Sci. 49:41-45.

Adsule, R.N., S.S. Kadam and H.K. Leung. 1989. Lentil. pp. 133-152. In: Salunkhe and Kadam (ed.), CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization. Vol. II. CRC Press. Inc. Boca Raton, Florida.

Ahmad, T., H. Rahman and M.H. Laghari. 2004. Effect of dıfferent auxıns on *ın vıtro* rootıng of peach rootstock gf 677. Sarhad J. Agric. 20:373-375.

Bahl, P.N., S. Lal and B.M. Sharma. 1993. An overview of the production and problems in south east Asia. pp. 1-10. In: W. Erskine and M.C. Saxena (ed.). Lentil in south Asia proceedings of the seminar on lentils in south Asia. ICARDA, Aleppo, Syria.

Bajaj, Y.P.S. and M.S. Dhanju. 1979. Regeneration of plants from apical meristem tips of some legumes. Curr. Sci. 84:906-907.

Bennet-Clark, T.A and N.P**.** Kefford. 1953**.** Chromatography of the growth substances in plant extracts. Nature 171**:**645**.**

Christou, P. 1994. The biotechnology of crop legumes. Euphytica 74:165-185.

De Klerk, G.J., W.V.D. Krieken and J.C. De Jong. 1999. The formation of adventitious roots: new concepts, new possibilities. *In Vitro* Cell. Dev. Biol-Plant 35:180-199.

Fratini, R. and M.L. Ruiz. 2002. Comparative study of different cytokinins in the induction of morphogenesis in lentil (*Lens culinaris* Medik.). *In Vitro* Cell. Dev. Biol-Plant 38:46–50.

Fratini, R. and M.L. Ruiz. 2003. A rooting procedure for lentil (*Lens culinaris* Medik.) and other hypogeous legumes (pea, chickpea and *Lathyrus*) based on explant polarity. Plant Cell Rep. 21:726-732.

Goszczynska, D., R.M. Rudnicki and M.S. Reid. 1985. The role of plant hormones in the postharvest life of cut flowers. Acta Hort. 167:79-93.

Khawar, K.M. and S. Özcan. 2002a. High frequency shoot regeneration from cotyledonary node explants of different lentil (*Lens culinaris* Medik) genotypes and *in vitro* micrografting. Biotechnol & Biotechnol Eq. 16:12-17.

Khawar, K.M. and S. Özcan. 2002b. Effect of indole-3-butyric acid on *in vitro* root development in lentil (*Lens culinaris* Medik.). Turk. J. Bot. 26:109-111.

Khawar, K.M., C. Sancak, S. Uranbey and S. Ozcan. 2004. Effect of Thidiazuron on shoot regeneration from different explants of lentil (*Lens culinaris* Medik.) via organogenesis. Turk. J. Bot. 28:421-426.

Krieken, W.V.D., H. Breteler, M.H.M. Visser and D. Mavridou. 1993. The role of the conversion of IBA into IAA on root regeneration in apple: Introduction of a test system. Plant Cell Rep. 12:203-206.

Lawton, K.A., K.G. Raghothama, P.B. Goldsbrough and W.R. Woodson. 1990. Regulation of senescence-related gene expression in carnation flower petals by ethylene. Plant Physiol. 93:1370-1375.

Leopold, A.C. 1955. Auxins and plant growth. Univ. of Calif. Press, Berkeley and Los Angeles.

Ludwig-Mtiller, J., S. Sass, E.G. Sutter, M. Wodner and E. Epstein. 1993. Indole-3-butyric acid in *Arabidopsis thaliana*: identiJication and quantification. Plant Growth Regul. 13:179-187.

Malik, K.A. and P.K. Saxena. 1992. Thidiazuron induces high frequency shoot regeneration in intact seedlings of pea (*Pisum sativum*), chickpea (*Cicier arieti*num) and lentil (*Lens culinaris*). Aust. J. Plant Physiol. 19:731–740.

Mallick, M.A. and A. Rashid. 1989. Induction of multiple shoots from cotyledonary node of grain legumes, pea and lentil. Biol. Plant. 31:230-232.

Molnar, Z., B. Jenes and V. Ördog. 1999. Genetic transformation of pea (*Pisum sativum* L.) via particle bombardment. Recent Advances in plant biotechnology. September 4-11, Stara Lesna, Slovak Rpublic 153:141–145.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15:473-497.

Nordstrom, A.C., F.A. Jacobs and L. Eliasson. 1991. Effect of exogenous indole-3-acetic acid and indole-3- butyric acid on internal levels of the respective auxins and their conjugation with aspartic acid during adventitious root formation in pea cuttings. Plant Physiol. 96:856-861.

Polanco, M.C., M.I. Pelaez and M.L. Ruiz. 1988. Factors affecting callus and shoot formation from *in vitro* cultures of *Lens culinaris* Medik. Plant Cell Tiss. Org. Cult. 15:175-182.

Polanco, M.C. and M.L. Ruiz. 1997. Effect of benzylaminopurine on *in vitro* and *in vivo* root development in lentil (*Lens culinaris* Medik.). Plant Cell Rep. 17:22–26.

Polanco, M.C. 2001. Factors that affect plant regeneration from *in vitro* culture of immature seeds in four lentil genotypes. Plant Cell Tiss. Org. Cult. 66:133-139.

Ruzicka, A., K. Luing, S. Vanneste, R. Podhorska, T. Beeckman, J. Friml and E. Benkova. 2007. Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. Plant Cell. 19:2197–2212.

Sarker, R.H., B.M. Mustafa, A. Biswas, S. Mahbub, M. Nahar, R. Hashem and M.I. Hoque. 2003. *In vitro* regeneration in lentil (*Lens culinaris* Medik.). Plant Tissue Cult. 13:155-163.

Savage, G.P. 1998. The composition and nutritive value of lentils (*Lens culinaris*). Nutr. Abst. Rev. 5:319–343.

Saxena, P.K. and J. King. 1987. Morphogenesis in lentil: Plant regeneration from callus cultures of *Lens culinaris* Medik. via somatic embryogenesis. Plant Sci. Irish Republic. 52:223-227.

Sevimay, C.S., K.M. Khawar and E. Yuzbasioglu. 2005. Adventitious shoot regeneration from different explants of wild lentil (*Lens culinaris* subsp. orientalis). Biotechnol & Biotechnol Eq. 19:46-49.

Singh, R.K. and S.S. Raghuvanshi. 1989. Plantlet regeneration from nodal segment and shoot tip derived explant of lentil. Lens News Letter 16: 33-35.

Skutnik, E., A. Łukaszewska and K. Tyborowska. 1999. Retarding senescence of cut leaves of *Hosta plantaginea* by growth regulators. Ann. Warsaw Agricult. Univ. – SGGW, Horticul. Landsc. Architect. 20:3-8.

Snedecor, G.W. and W.G. Cocharan. 1937. Statistical methods. The Iowa State University Press. Iowa. USA.

Surekha, C., M.R. Beena, A. Arundhati, P.K. Singh, R. Tuli, A. Dutta-Gupta and P.B. Kirti. 2005. Agrobacterium-mediated genetic transformation of pigeon pea (*Cajanus cajan* (L.) Millsp.) using embryonal segments and development of transgenic plants for resistance against *Spodoptera.* Plant Sci. 169:1074-1080.

Swarup, R., G. Parry, N. Graham, T. Allen and M.J. Bennett. 2002. Auxin cross-talk: integration of signaling pathways to control plant development. Plant Mol. Biol. 49:411–426.

Van Kessel, C. 1994. Seasonal accumulation and partitioning of nitrogen by lentil. Plant Soil 164:69–76.

Warkentin, T.D. and A. McHughen. 1993. Regeneration from lentil cotyledonary nodes and potential of this explant for transformation by *Agrobacterium tumefaciens.* Lens News Letter 20:26-28.

Weaver, R.J. 1972. Plant growth substances in agriculture. 1st ed. WH Freeman and Co., San Francisco, USA.

Wiesman, Z., J. Riov and E. Epstein. 1988. Comparison of movement and metabolism of indole-3-acetic acid and indole-3-butyric acid in mung bean cuttings. Physiol. Plant. 74:556–560.

Williams, D.J. and A. McHughen. 1986. Plant regeneration of the legume *Lens culinaris* Medik. (lentil) *in vitro*. Plant Cell Tiss. Org. Cult. 7:149-153.

Woodson, W.R. and K.A. Lawton. 1988. Ethylene induced gene expression in carnation petals: Relationship to autocatalytic ethylene production and senescence. Plant Physiol. 87:498-503.

Xu, J., H. Hofhuis, R. Heidstra, M. Sauer, J. Friml and B. Scheres. 2006. A molecular framework for plant regeneration. Sci. 311:385-388.

Zahran, H.M. 1999. Rhizobium-Legume symbiosis and nitrogen fixation under severe conditions in arid climate. Microbiol. Mol. Biol. Rev. 63:968-989.