Pak. J. Bot., 43(2): 1283-1293, 2011.

SOMATIC EMBRYOGENESIS AND SHOOT REGENERATION INDUCED IN CUCUMBER LEAVES

MUHAMMAD USMAN*, ZAHOOR HUSSAIN AND B. FATIMA

Institute of Horticultural Sciences, University of Agriculture, Faisalabad-Pakistan
**Corresponding author: musman74@gmail.com*

Abstract

Commercial cucumber cultivars were explored for embryogenesis and plant regeneration induced in somatic tissues on plant growth regulators (PGRs). Maximum callus induction 94.16% and 76% was observed in leaf disc explants on MS medium supplemented with 2,4-D (2 mgL^{-1}), NAA and BAP (1.5 mgL^{-1}, each), respectively. Seed cotyledon explants induced maximum calli (77%) on 4.0 + 0.75 mgL^{-1} (BAP + NAA, respectively). Calli induced in leaf disc on the highest level of 2,4-D (5 mgL^{-1}) yielded the highest embryo formation (23%) whereas calli induced on BAP and BAP + NAA (5 + 1 mgL^{-1}) regenerated into 14% and 12%, shoots respectively. These shoots were excised and rooted on MSO medium. The plantlets were transplanted in pots and transferred to field after acclimatization. The developed plant material will be morphologically and genetically characterized for homozygosity.

Introduction

Micropropagation and shoot regeneration protocols for cucumber are required to decrease the cost of hybrid seed production which is usually higher than 30% of total seedling cost (Konstas & Kintzios, 2003). Regeneration of cucumber plants have been reported with limited success either directly or indirectly on various explants (Ziv, 1992). These include anthers (Lazarte & Sasser, 1982), cotyledons (Trulson & Shahin, 1986), leaves (Malepszy & Nadolska-Ocyzyk, 1983), protoplasts (Jia *et al*., 1986), axillary bud explants (Handley & Chambliss, 1979), shoot tip culture (Vasudevan *et al*., 2004; 2008), nodal segments (Ahmad & Anis, 2005) and embryonal axis (Vasudevan *et al*., 2007). Poor development of embryos, low differentiation of callus into shoots and poor survival rate of plants after acclimatization has been reported earlier (Ziv & Gadasi, 1986; Kim *et al*., 1988; Ziv, 1992). Regeneration via direct organogenesis is usually preferred for commercial mass propagation of vegetable crops. Much work on organogenesis of cucumber (*Cucumis sativus* L.) have been difficult to repeat, results have been unpredictable and the plant population regenerated *in vitro* showed variability. Maciejewska *et al*., (1972) were the first to report organogenesis from callus produced by stem pieces of cucumber, but they did not describe methods for shoot regeneration. Alsop *et al*., (1978) obtained only callus from several organ explants with various concentrations of NAA and BAP, each. However, some bud-like knobs were observed in callus grown at 0.1mgL^{-1} NAA and BAP. Aziz *et al*., (1986) also described bud-like nodules on callus derived from internode pieces of cucumber, but they could induce root formation only. Other workers have been able to produce adventitious buds and shoots from both hypocotyls and cotyledons (Novak & Dolezeloa, 1982). Cotyledons appear to be better explant for use in organogenic experiments (Moreno *et al*., 1985). However, callus from cotyledons was characterized by proliferation of fibrous roots whereas callus derived from hypocotyls did not

(Novak & Dolezeloa, 1982). The present study was initiated to optimize efficient regeneration of cucumber plants from embryogenic calli using various somatic tissues as explants on different auxins and cytokinins alone and in combination.

Materials and Methods

Explant sources and sterilization procedures: The plant parts excised and used as explant for further propagation of commercial cucumber cultivars Bethalpha and Marketmore include 1) seed, 2) cotyledon (without embryonic axis) 3) cotyledon leaf and 4) leaf disc. Seeds and cotyledons were surface disinfected with 70% ethanol for three minutes followed by thorough rinsing with sterilized distilled water thrice. Seeds were kept in 5% sodium hypochlorite solution for five minutes followed by three rinses with sterilized distilled water.

Explant isolation and culture procedures: De-coated seeds of both the cultivars of cucumber were cultured on Murashige & Skoog (1962) medium for germination and data were colleted for germination percentage. Stem cuttings and shoot tips (3-5 mm in length) excised from *in vitro* raised seedlings were sub-cultured on the same medium in 250 ml glass vessels for development of complete plants. Cotyledons, cotyledon leaves, excised from seedlings at two leaf stage and leaf disc, excised from micropropagated plants, were cultured in modified MS media for callus induction and plant regeneration.

Media composition and sterilization: Murashige & Skoog (1962) basal medium was used for seed germination and micropropagation of cucumber. It was modified for embryogenesis and regeneration of cucumber plants with different concentrations of 2, 4-D (1, 2, 3 and 5 mgL^{-1}), BAP and NAA separately (0.2, 0.4, 0.6, 1.0 and 1.5 mgL^{-1}) and in combinations (2.0+0.25, 3.0 + 0.5, 4.0 + 0.75 and 5.0 + 1.0 mgL^{-1}, respectively). Media were sterilized in an autoclave for 20 minutes at 121°C temperature and 15 psi pressure.

Culture conditions: After inoculation, the cultures were placed in the growth room maintained at temperatures 25 ± 2°C and facilitated with 2500 lux of light intensity.

Experimental layout: The experiments were laid out in completely randomized design (CRD) with at least three replications. Twenty five tubes were used per treatment. Data were collected on percentage basis, analyzed and the means were compared for Least Significance Difference (Steel & Torrie, 1980).

Results and Discussion

1. Seed germination and plant multiplication: Seeds of cucumber cultivars viz. Bethalpha and Marketmore responded positively for germination *in vitro* when cultured on MS medium. Bethalpha and Marketmore cultivar seeds 28% and 24% germinated, respectively, on the 2nd day of culture and 100% germination percentage was obtained in 12-15 days of culture (date not shown). Seed germination and plant multiplication using node and shoot tip culture was found better in Bethalpha compared to Marketmore, however, the results were non-significantly different to each other (Fig. 1). The findings are in accordance with the results of Dong & Jia (1991) and Chee (1991) for germination and multiplication behavior of cucumber cultivars over the time.

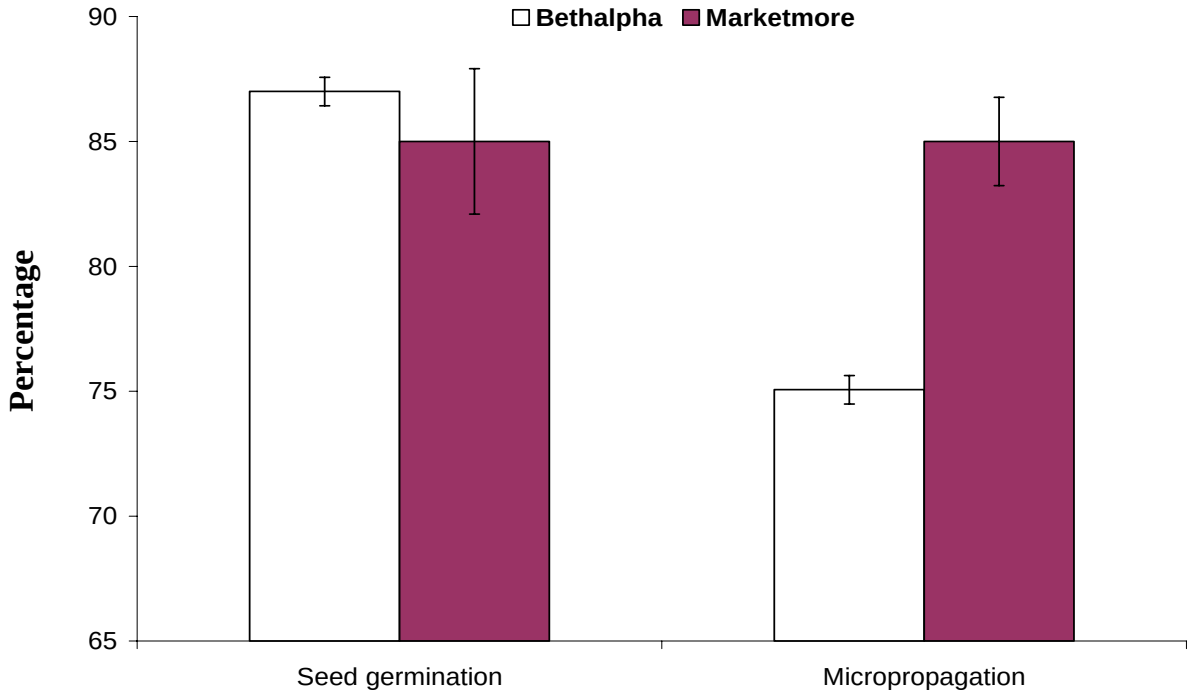

Fig. 1. Frequency of seed germination and micropropagation on MS medium in cucumber cultivars. Data are means of three Reps.

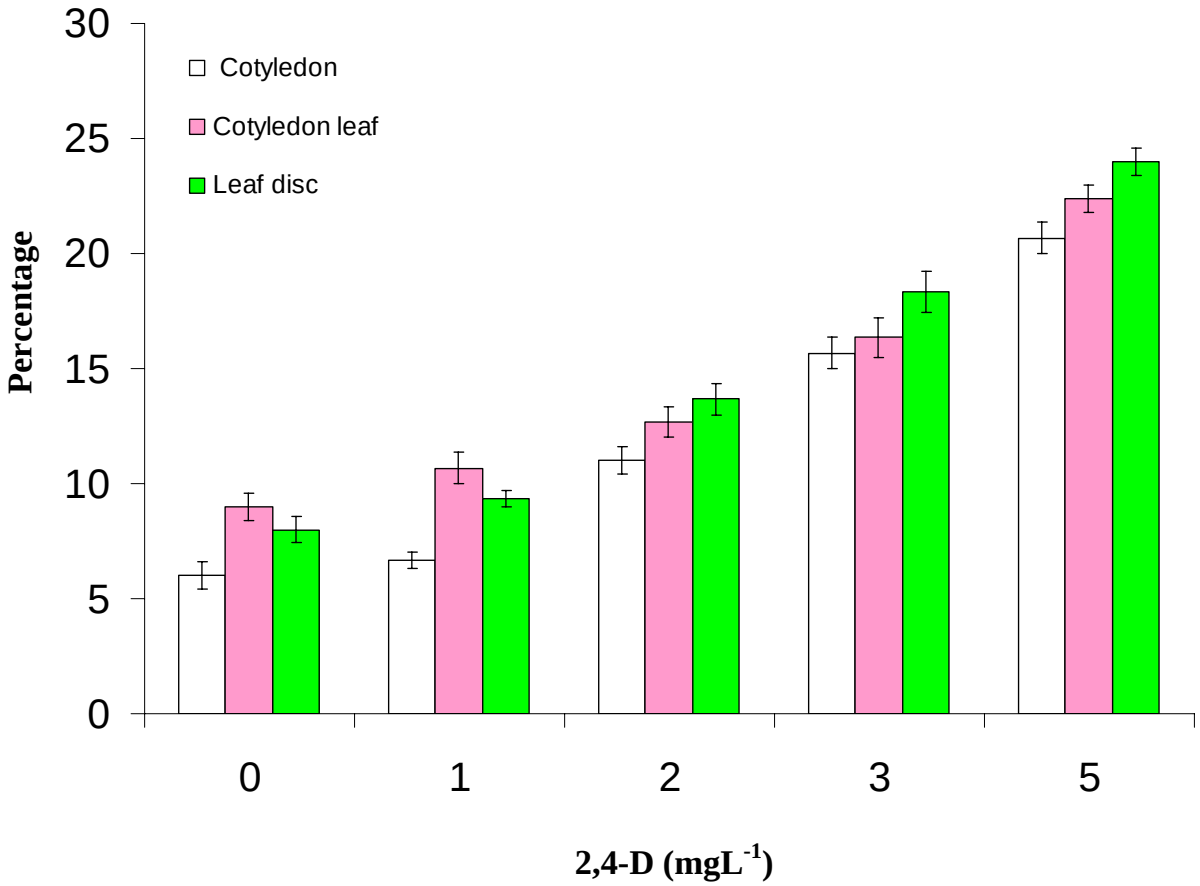

Fig. 2. Embryogenesis on MSO medium in calli induced from different explant sources on MS medium containing 2,4-D in cucumber . Data are means of three Reps. and vertical bars (I) show Standard Error

2. Callus induction and proliferation behavior: Different explants from cucumber cultivars were explored for callus induction response on different media, however, no significant genotypic differences were observed (data not shown). On MS medium containing 2,4-D increase in the concentration of 2,4-D significantly increased callus induction percentage in both leaf disc and cotyledon leaf explants (94.16% and 87.00%, respectively), however, in case of seed cotyledon, callus induction was maximum (89.50%) at 2 mgL^{-1} 2,4-D and further increase in 2,4-D showed decline in callus induction percentage (Table 1). Similar trend was observed when these explants were cultured on medium containing either BAP or NAA for callus induction @1.5 mgL^{-1} and the highest callus induction was observed in leaf disc explant (76.00%) on both media. In case of BAP + NAA, the highest levels of both PGRs induced maximum callus induction in seed cotyledon explant (77%) compared to cotyledon leaf explant (64%). In leaf disc explants, maximum callus induction (54%) was observed on 4.0 + 0.75 mgL^{-1} of BAP + NAA, respectively, and further increase in the PGRs showed decline in callus induction. Overall among different explants explored for callus induction on different media, the highest callus induction was observed in leaf disc explant followed by cotyledon and cotyledon leaf explant on MS medium containing 2,4-D (Table 1).

Table 1. Callus induction percentage in different explants on MS medium containing different Plant Growth Regulators (PGRs).

PGR Treatments	Cotyledon	Cotyledon Leaf	Leaf Disc
2,4-D			
Control	62.66 e	65.16 e	69.83e
1.0 mgL^{-1}	71.50 d	73.83 d	73.50d
2.0 mgL^{-1}	89.50 a	76.83 c	86.50c
3.0 mgL^{-1}	82.00 c	80.33 b	91.33b
5.0 mgL^{-1}	86.66 b	87.00 a	94.16a
LSD values	1.39	1.94	2.13
BAP			
Control	56.50 bc	40.00d	39.17e
0.2 mgL^{-1}	60.00 ab	45.00c	66.67b
0.4 mgL^{-1}	56.00 bc	44.00 cd	60.67c
0.6 mgL^{-1}	52.00 c	44.00 cd	60.00c
1.0 mgL^{-1}	64.00 a	52.00 b	48.67d
1.5 mgL^{-1}	64.00 a	64.00 a	76.50a
LSD values	4.76	4.61	3.93
NAA			
Control	54.50 bc	45.00 c	38.33 c
0.2 mgL^{-1}	58.00 ab	48.00 bc	72.33 a
0.4 mgL^{-1}	54.00 bc	52.00 b	64.33 b
0.6 mgL^{-1}	49.00 c	40.00 d	72.83 a
1.0 mgL^{-1}	63.00 a	48.00 bc	60.33 b
1.5 mgL^{-1}	63.00 a	64.00 a	76.00 a
LSD values	4.70	4.61	4.37
BAP+NAA			
Control	69.00 b	39.67 c	32.33 c
2.0 + 0.25 mgL^{-1}	68.00 b	52.00 b	46.67 b
3.0 + 0.50 mgL^{-1}	76.00 a	40.00 c	46.67 b
4.0 + 0.75 mgL^{-1}	65.00 b	52.00 b	54.17 a
5.0 + 1.0 mgL^{-1}	77.00 a	64.00 a	48.67 b
LSD values	4.82	4.46	3.11

Means sharing the same letters in columns are statistically non-significant.

Data are means of three reps. from two independent experiments with 20 explants per treatment per rep.

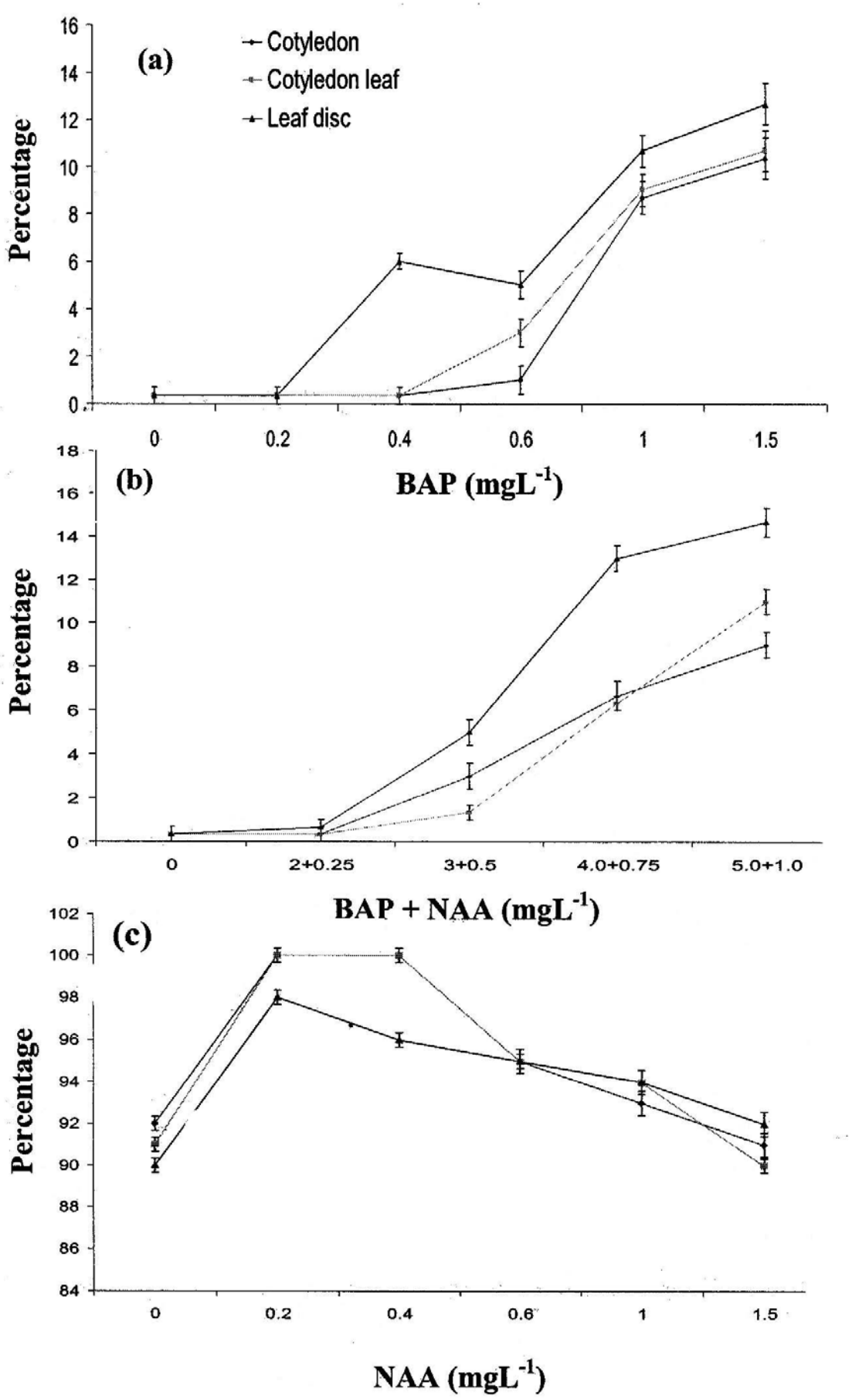

Fig. 3a-c. Shoot regeneration percentage in cotyledon, cotyledon leaf and leaf disc explants on MS medium containing different levels of NAA (a), BAP (b) and NAA + BAP (c).

Significantly greater calli were induced on various levels of 2,4-D, BAP and BAP in combination with NAA. Our results are in accordance with the findings of Kim *et al*., (1988) and Ali *et al*., (1991) who induced callus from cotyledon of Burpless hybrid cucumber and each cotyledon was divided in 2,4,6 and 24 pieces. The findings of Garcia-Sogo (1990), Lou & Kako (1994) and Ladyzynski *et al*., (2001) who developed an efficient method for obtaining callus from cotyledon, hypocotyls and first leaves in cucumber are in agreement as significantly higher calli induced on higher levels of 2,4-D. However, our results are in conformity with the findings of Alsop *et al*., (1978) and Punja *et al*., (1990) who observed callus induction from cotyledon explant. Our findings are further supported by the observations of Gambley & Dodd (1990) who obtained callus from cv. Crystal Salad hybrid cucumber. However, our results are contrary to the findings of Punja *et al*., (1990) and Gambley & Dodd (1990) who obtained genotypic differences for callus induction from cv. Crystal Salad hybrid cucumber and this may be attributed to the use of different genotypes for callus induction.

4. Embryogenesis: Calli derived from cotyledon (without embryonic axis), cotyledon leaf and leaf disc explants were carefully excised and sub-cultured on MS medium supplemented with different levels of 2,4-D, NAA, BAP and NAA + BAP. Calli obtained from all the explants showed callus proliferation on 2,4-D while rest of the PGRs showed organogenesis upon sub-culturing of calli (Fig. 4D,E). When the calli proliferated on 2,4-D were placed as small chunks on control (MSO) medium, it showed embryogenesis. The highest embryo formation (23%) occurred in embryogenic calli derived of leaf disc followed by cotyledon and cotyledon leaf explants on 2,4-D (5.0 mgL^{-1}) while the lowest embryogenesis was observed on MS medium devoid of growth hormones and lower concentration of 2,4-D (1.0 mgL^{-1}) as shown in Fig. 2. After maturity obtained on the same medium, somatic embryos were germinated on MSO medium for plant regeneration.

Auxins are well known for the induction of embryogenesis alone or in combination particularly 2,4-D (Tabei *et al*., 1991). Callus induction on auxin containing media and embryogenesis on auxin free medium is being used as standard method in a wide range of plant species (Fujimora & Komamin, 1974; Raghavan, 2004). Earlier reports of embryogenic callus induction in cotyledon and leaf disc explants in cucurbits like squashes and melons on 2,4-D (Noel *et al*., 1992) are strengthening our results showing embryogenic callus induction in both explants on 2,4-D in cucumber. Our findings of development and maturation of somatic embryos in 2,4-D induced calli in cucumber somatic tissues on transfer to auxin free medium are supported by similar reports in *Cucurbita moschata* (Kwak & Fujeida, 1988) and carrot (Feher *et al*., 2003). Removal of auxin, 2,4-D in this case, checks exogenous availability of the auxin and decreases the endogenous level of IAA, thus limiting callus proliferation leading to establishment of pre-globular embryos, that develop later on the same medium as observed in carrot (Yang *et al*., 2004; Kikuchi *et al*., 2005; Ogata *et al*., 2005). These findings are in conformity with our results of somatic embryogenesis in cucumber. Earlier reports of Jelaska (1986) and Noel *et al*., (1998) described no negative effects of cytokinins on somatic embryogenesis in squashes in contrast we found no embryogenesis in calli induced on BA showing inhibitory effect. Similar to our results, pretreatment with either auxin or a cytokinin was not necessary for embryo initiation in squashes (Kintzios *et al*., 2002). We obtained embryogenically competent calli without any pretreatment at higher levels of 2,4-D as reported earlier in melon (Tabei *et al*., 1991), pepper (Kintzios *et al*., 1996) and cucumber (Kujipers *et al*., 1996).

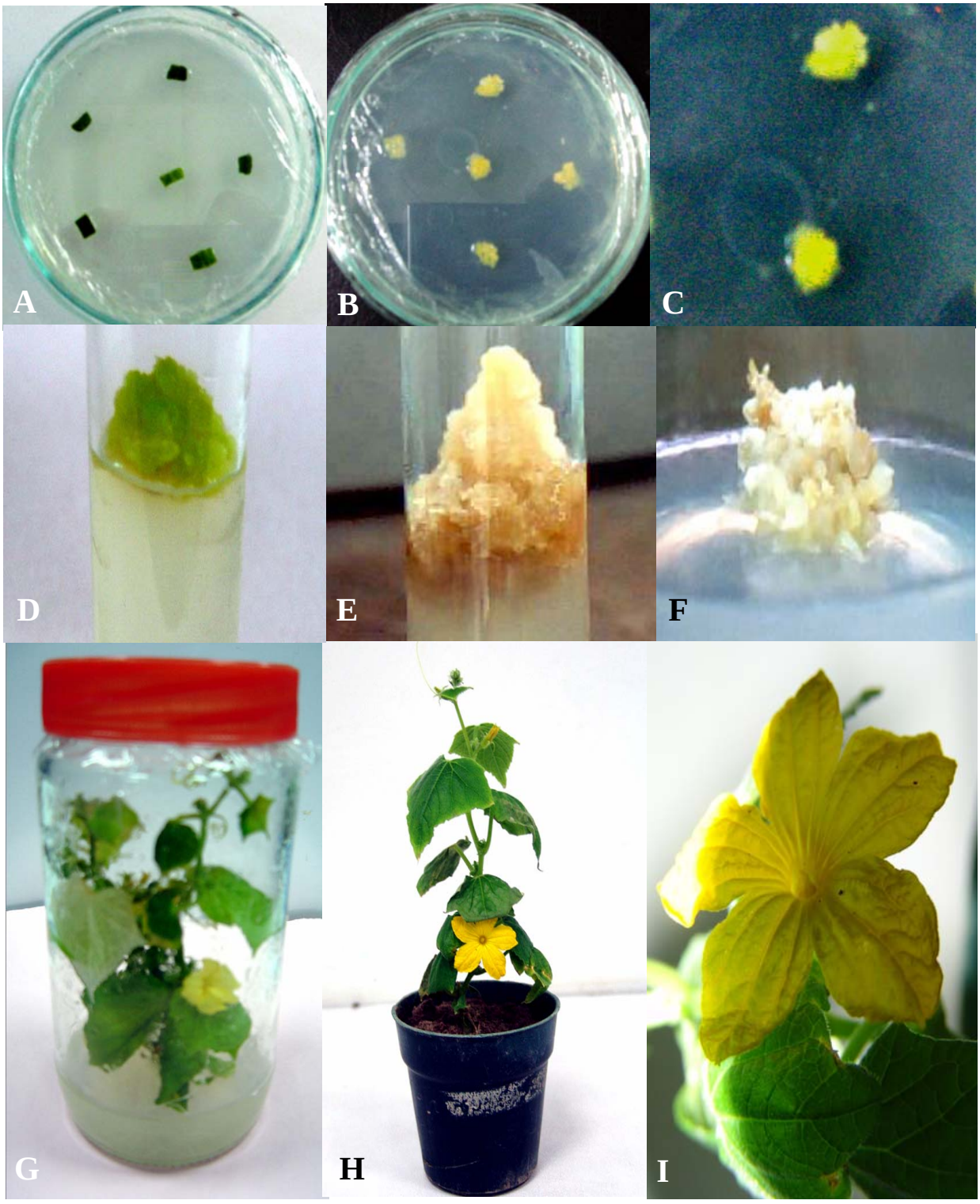

Fig. 4. Callus induction and proliferation (B-E) response after 4 weeks of culture on MS medium containing 2,4-D and BAP respectively and plant regeneration on MSO medium (G). Figure shows leaf disc explants cultured on BAP for shoot induction (A), nodular structures developed in calli on BAP (B) that later regenerated into shoots and development of nodular structures that later developed into roots in calli on NAA (F). Fertile regenerated plant (H) is showing classical early maleness where fig. C & I are close ups of proliferating calli and flower after anthesis.

5. Organogenesis: Calli induced on BAP and BAP + NAA media showed shoot regeneration in all the three explants used. Shoot regeneration increased with increase in level of both PGRs and maximum shoot regeneration (14%) was observed in leaf disc explant induced calli on $5mgL^{-1}$ + 1 mgL^{-1}, respectively, followed by BAP showing 12% shoot induction (Fig., 3a,b; 4B,C). In other explants, cotyledon leaf explant showed better regeneration than cotyledon explant in BAP and BAP + NAA media and the highest values (10%) were observed on the highest levels of these PGRs followed by cotyledon explant showing shoot regeneration on BAP. The regenerated shoots were excised and rooted on MSO medium. Calli induced on NAA showed root induction and the highest frequency (100%) was observed in cotyledon and cotyledon leaf explant induced calli on the lowest level of NAA used (0.2 and 0.4 mgL^{-1}) and rooting response decreased with increase in level of NAA in the medium (Fig., 4F) and this calli did not develop shoots.

Our findings regarding shoot regeneration on BAP are in conformity with report of Han *et al*., (2004) who concluded BA as an essential factor for shoot regeneration in bottle gourd. Similar conclusion has been drawn in another study on bottle gourd stating BA essential for shoot bud formation (Saha *et al*., 2007). Use of cytokinins in combination with auxins are reported to induce shoot buds as we have observed in case of all the three somatic explants using BA in combination with NAA. Similar results have been reported in *Capsicum* using BA and IAA in cotyledon and leaf explants (Venkataiah *et al*., 2001). Efficient shoot induction was observed by Khan *et al*., (2006) in tomato leaves on higher levels of BAP combined with lower levels of NAA. These results are similar to our findings regarding shoot induction in leaf disc explant, cytokinins and its combination with auxin as BAP/NAA in cucumber. In another report on tomato, regeneration has been significantly higher in MS medium containing IAA and BAP combined with GA_3 (0.5, 1.5 and 2 mgL^{-1}, respectively) compared to medium with GA_3 (Afroz *et al.*, 2009). Our findings regarding subculturing nodular calli on the same medium (BAP) for shoot development is in agreement with findings of Seo *et al*., (2000) in terms of shoot induction from leaf explant induced calli in cucumber at much lower concentration of BAP combined with higher levels of NAA. We differ, however, in the levels of BAP/NAA and cucumber cultivars used for this purpose. Moreover our results of callus induction on NAA in cotyledon, cotyledon leaf and leaf disc explants with no shoots rather root induction are also supported by similar findings in calli on lower levels of NAA with no or 0.1 µM BAP showing zero shoot formation. It is concluded that only 2,4-D induced calli was found to be embryogenic in nature and regenerated in to somatic embryos on transfer to MSO medium. The calli induced on BAP and NAA+BAP was non-embryogenic and regenerated into shoots on subculturing calli on medium containing same PGR. Leaf disc explant was found better for somatic embryogenesis and shoot regeneration.

We report here a two way plant regeneration system using somatic tissues in commercial cucumber cultivars. Regenerated plants from both sources were transplanted in pots containing sterilized soil for acclimatization and transplanted in the field to observe their fertility status. In future, the morphological and genetic characterization of this germplasm will be made to confirm extent of homozygosity in the regenerated plants. Leaf explants are easily available in abundance and could be multiplied by simple micropropagation, thus becoming much better explant compared to either cotyledon or cotyledon leaf which is available in less number and for a short duration. Our results of

developing embryogenic calli by significantly higher shoot induction compared to other explants make this protocol favorable for efficient multiplication of cucumber plants. Such protocols could be helpful for getting regeneration in transformed tissues on selective medium for developing transgenic plants in this economically important member of Cucurbitaceae family as well.

References

Ahmad, N. and M. Anis. 2005. *In vitro* Mass Propagation of *Cucumis sativus* L., from Nodal Segments. *Turk. J. Bot.*, 29: 237-240.

Afraz, A., Z. Chaudhry, R. Khan, H. Rashid and S.A. Khan. 2009. Effect of GA_3 on regeneration response of three tomato cultivars (*Lycopersicon esculentum*). *Pak. J. Bot*., 41: 143-151.

Ali, N., R.M. Skirvin and W.E. Splittstesser. 1991. Regeneration of *Cucumis sativus* L. from cotyledons of small explants. *HortScience*, 26: 925.

Alsop, W.R., W.W. Cure, G.F. Evans and R.L. Mott. 1978. Preliminary report on *in vitro* propagation of cucumber. *Cucurbit Genet. Coop. Rept*., 1: 1-2.

Aziz, H.A., B.H. McCown and R.L. Lower. 1986. Callus initiation from cucumber (*Cucumus sativus* L.) fruits. *Cucurbit Genet. Coop. Rpt.*, 9: 3.

Chee, P.P. 1991. Somatic embryogenesis and plant regeneration of squash cucurbita pepo L. cv. YC 60. *Plant Cell Reports*, 9: 620-622.

Dong, J.Z. and S.R. Jia. 1991. High efficiency plant regeneration from cotyledons of watermelons (*Citrulus vulgaris* Schrad.). *Plant Cell Reports*, 9: 559-562.

Feher, A., T. Pasternak and D. Dudits. 2003. Transition of somatic plant cells to an embryogenic state. *Plant Cell Tissue & Organ Culture*, 74: 201-228.

Fujimura, T. and A. Komamine. 1979. Synchronization of somatic embryogenesis in a carrot suspension culture. *Plant Physiol.*, 64: 162-164.

Gambley, R.L. and W.A. Dodd. 1990. An *in vitro* technique for the production de novo of multiple shoots in cotyledon explants of cucumber (*Cucumber sativus* L.). *Plant Cell, Tissue and Organ Culture*, 12: 67-74.

García-Sogo, B. 1990. *Morfogénesis en cultivo in vitrode melón: regeneración de plantas con alta eficacia a partir de células y protoplastos*. PhD Thesis, Facultad de Ciencias Biológicas, Universidad de Valencia, Spain.

Han, J.S., D.G. Oh, I.G. Mok, H.G. Park and C.K. Kim. 2004. Efficient plant regeneration from cotyledon explants of bottle gourd (*Lagenaria siceraria* Standl.). *Plant Cell Reports*, 23: 291-296.

Handley, L.W. and O.L. Chambliss. 1979. *In vitro* propagation of (*Cucumus sativus L*) *HortScience*, 14:22-23.

Jelaska, S. 1986. Cucurbits. In: Bajaj YPS (Ed) *Biotechnology in agriculture and forestry* 2. Crops. Springer, Berlin Heidelberg New York, pp 371-386.

Jia, S.R., Y.Y. Fu and Y. Lin. 1986. Embryogenesis and regeneration from cotyledon protoplast culture of cucumber (*Cucumis sativus* L.). *Plant Physiol.*, 124:393-398.

Khan, M.S., M. Usman and M.I. Lilla. 2006. Facile plant regeneration from tomato leaves induced with spectinomycin. *Pak. J. Bot*., 38: 947-952.

Kikuchi, A., N. Sanuki and K. Higashi. 2005. Abscisic acid and stress treatment are essential for the acquisition of embryogenic competence by carrot somatic cells. *Planta*, DOI 10.1007/s004425-005-0114-y.

Kim, S.G., J.R. Chang, H.C. Cha and K. Lee. 1988. Callus growth and plant regeneration in diverse cultivars of cucumber *(Cucumis sativus L.). Plant Cell, Tissue and Organ Culture*, 12: 67-74.

Kintzios, S., J. Drossopoulos, M. Manousaridou and C.D. Holevas. 1996. Competence for callus induction on mature pepper leaves depends upon specific developmental stages of the donor leaves. *Sci. Hort.*, 65: 341-347.

Kintzios, S.E. P. Sereti, P. Bluchos, J.B. Drossopoulos, C.K. Kitsaki and A.L. Tasakalidis. 2002. Growth regulator pretreatment improves somatic embryogenesis from leaves of squash (*Cucurbita pepo* L.) and melon (*Cucumis melo* L.). *Plant Cell Reports*, 21: 1-8.

Konstas, J. and S. Kintzios. 2003. Developing a scale up system for the micropropagation of cucumber (*Cucumis sativus* L.) the effect of growth retardants, liquid culture and vessel size. *Plant Cell Reports*, 21: 538-548.

Kuijpers, A.M., H. Bouman and G.J. De Klerk. 1996. Increase of embryogenic callus formation in cucumber by initial culture on high concentration of 2, 4-dichlorophenoxyacetic acid. *Plant Cell Tissue & Organ Culture*, 46: 81-83.

Kwack, S.N. and K. Fujieda. 1988. Somatic embryogenesis in cultured unfertilized ovules of *Cucurbita moschata*. *J. Jpn. Soc. Hort. Sci.*, 57: 34-42.

Ladyzynski, M., A. Korzeniewska and S. Malepszy. 2001. Recurrent regeneration through somatic embryogenesis reduces yield in cucumber. *HortScience*, 36(5): 987.

Lazarte, J.E. and C.C. Sasser. 1982. Asexual embryogenesis and plantlet development in anther culture of *Cucumus sativus* L. *HortScience*, 17: 88.

Lou, H. and S. Kako. 1994. Somatic embryogenesis and plant regeneration in Cucumber. *HortScience*, 8: 906-909.

Maciejewska, P.W., A. Rennert and E. Milewska. 1972. Callus induction and growth of tissue culture derived from cucumber plant organs of four different sex types. *Acta. Soc. Bot. Poland*., 41: 329-339.

Malepszy, S. and A. Nadolska-Ocyzyk. 1983. *In vitro* culture of (*Cucumis sativus* L.) Regeneration of plantlet from callus formed by leaf explants. *Z. Pllanzenphysiol*, 111: 273-276.

Moreno, V., M. Garcia-Sogo, I. Granell, B. Garcia-Sogo and L.A. Roig. 1985. Plant regenerations from calli of melon (*Cucumus melo* L.) cv. Amarillo Org. *Plant Cell Tissue & Organ Culture*, 5: 139-146.

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

Noel, M., D. Comeau and P. Lenee. 1992 Embryogenese somatique de la courgette (*Cucurbita pepo* L.) a partir de cotyledons. In: Comptes-rendus du ler forum jeunes chercheurs de l'IAPTC –'Du gene a l'entreprise'. Amiens, France, pp. 14-18.

Novak, F.J. and M. Dolezeloa. 1982. Hormone control of growth and differentiation in the *in vitro* cultured tissue of cucumber (*Cucumis sativus* L.). *Biologia*, 37: 283-290.

Ogata, Y., M. Lizuka, D. Nakayama, M. Ikeda, H. Kamada and T. Koshiba. 2005. Possible involvement of abscissic acid in the induction of secondary somatic embryogenesis on seed coat-derived carrot somatic embryos. *Planta*, 22: 417-423.

Punja, Z.K., N. Abbas, G.G. Sarmento and F.A Tang. 1990. Regeneration of *Cucumis sativus* L. var. *sativus* and C. *sativus* var. hardwicki, *C. melo* and *C. mettuliferus* from explants through somatic emberyogenesis and organogenesis. *Plant Cell, Tissue & Organ Culture*, 21: 93-102.

Raghavan, V. 2004. Role of 2,4-dichlorophenoxyacetic acid (2,4-D) in somatic embryogenesis on cultured zygotic embryos of *Arabidopsis*: cell expansion, cell cycling, and morphogenesis during continuous exposure of embryos to 2,4-D. *Am. J. Bot.*, 91: 1743-1756.

Saha, S., H. Mori and K. Hattori. 2007. Synergistic effect of kinetin and Benzyl Adenine plays a vital role in high frequency regeneration from cotyledon explants of Bottle Gourd (*Lagenaria siceraria*) in relation to Ethylene production. *Biotech. Science*, 57: 197-202.

Seo, S.H., D.G. Bai, H.Y. Park. 2000. High frequency shoot regeneration from leaf explants of cucumber. *Plant Biotech.*, 1: 51-54.

Steel, R.G.D. and H.H. Torrie. 1980. *Principles and procedures for statistics. A biometrical approach*. McGraw Hill Book Company, New York.

Tabei, Y., T. Kanno and T. Nishio. 1991. Regulation of organogenesis and somatic embryogenesis by auxin in melon (*Cucumis melo* L.). *Plant Cell Reports*, 10225-229.

Trulson, A.J. and E.A. Shahin. 1986. *In vitro* plant regeneration in the genus *Cucumis*. *Plant Science*, 47: 35-43.

Vasudevan, A., N. Selvaraj, A. Ganapathi, C.W. Choi, M. Manickavasagam and S. Kasthurirengan. 2007. Direct plant regeneration from cucumber embryonal axis. *Biol. Plant*., 3: 521-524.

Vasudevan, A., N. Selvaraj, A. Ganapathi, S. Kasthurirengan, V. Ramesh Anbazhagan and M. Manickavasagam. 2004. Glutamine: a suitable nitrogen source for enhanced shoot multiplication in *Cucumis sativus* L. *Biol. Plant.,* 3: 125-128.

Vasudevan, A., N. Selvaraj, A. Ganapathi, S. Kasthurirengan, V. Ramesh Anbazhagan, M. Manickavasagam and C.W. Choi. 2008. Leucine and spermidine enhance shoot differentiation in cucumber (*Cucumis sativus* L.). *In vitro Cell. Dev. Biol.-Plant.* 44: 300-306.

Venkataiah, P., T. Christopher and K. Subhash. 2001. Plant regeneration and *Agrobacterium* mediated genetic transformation in four *Capsicum* species. *Capsicum and Eggplant News Letter,* 20: 68-71.

Venkataiah, P., T. Christopher and K. Subhash. 2003. Thiadizuron induced high frequency adventitious shoot formation and plant regeneration in *Capsicum annum* L. *Plant Biotech.,* 4: 245-250.

Yang, Z., L. Zhang, F. Diao, M. Huang and N. Wu. 2004. Sucrose regulates elongation of carrot somatic embryo radicals as a signal molecule. *Plant Mol. Biol.,* 54: 441-459.

Ziv, M. 1992. Micropropgation of *Cucumis* spp. In: (Eds.): Y.P.S. Bajaj. High – Ten and micropropagation. *Biotech. in Agriculture and Forestry,* Vol. 19. Springer, Berlin Heidelberg New York, pp. 72-90.

Ziv, M and G. Gadasi. 1986. Enhanced embryogenesis and plant regeneration from cucumber (*Cucumis sativus* L.) callus by the activated charcoal in Solid/Liquid double layer cultures. *Plant Sci.,* 47:115-122.

(Received for publication 4, January, 2010)