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Induced Mutation in Callus Cell Lines of *Daucus carota* L.

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Abstract: *Daucus carota* L. is an important vegetable food crop with high nutritive value. The changing climate, pollution, loss of vegetation has affected the crop quality and there is a need to improve the variety. So the present investigation was taken with an aim to induce mutation in callus cell lines for crop improvement. Callus induction from stem and leaf explants of *in vitro* grown *Daucus carota* L. plantlets was observed on 1X MS medium with 3% sucrose , 1% agar fortified with 2 mg L^{-1} 2,4-D. Mutation was induced in *Daucus carota* L callus cell lines by sodium azide and UV radiations. Callus cell lines grown on media containing 200 μM of sodium azide gave the best result with callus multiplication. Callus exposed at different time intervals under UV radiation showed multiplication of callus in all the exposures. Shoot induction was achieved for both the mutants on 2 mg L^{-1} BAP containing medium. The physiological estimation revealed that protein content (0.460±0.04 mg mL^{-1}) in 6 min UV exposed cell lines and carbohydrate content (0.820±0.08 mg mL^{-1}) in 4 min UV exposed cell lines are high when compared to control cell lines, sodium azide and other UV mutated cell lines. Mutation was confirmed through DNA fingerprinting.

Key words: *Daucus carota* L., sodium azide, UV radiation, callus cell lines, fingerprinting

INTRODUCTION

Daucus carota L. is an important member of family Apiaceae, it is valued as food mainly because it is rich source of the fat-soluble hydrocarbon, carotene ($C_{40}H_{56}$) the β form of which is the precursor of Vitamin A. The pigment from the roots are used for coloring butter and other food materials. It is used in treating kidney related diseases, dropsy, as nervine tonic, aphrodisiac stimulant and carminative (Pant and Manandhar, 2007). Genetic variability is fundamental to successful breeding programs in vegetatively and sexually propagated plants. This variation can occur naturally or can be induced through mutations, using physical, biological or chemical mutagens and has attracted the interest of plant breeders from many decades. Mutations have helped to produce many cultivars with improved economic value (Broertjes and van Harten, 1988) and study of genetics and plant developmental phenomena (Bulk *et al*., 1990; Bertagne-Sagnard *et al*., 1996). Mutation breeding as an alternative method to conventional plant breeding as a source of increasing variability and confer specific crop improvement without much altering its acceptable phenotype (Ojomo *et al*., 1979; Mensah and Obadoni, 2007). Physically and chemically induced mutation is a method to create genetic variation resulting in improved varieties with better characteristics (Wongpiyasatid *et al*., 2000; Arulbalachandran *et al*., 2009). Sodium azide is a chemical mutagen and is considered as one of the most powerful mutagen in plant (Mostafa, 2011). The successful utilization of sodium azide to generate genetic variability in plant breeding has been reported in *Helianthus annuus* L. (Mostafa, 2011) and other crops (Akhaury *et al*., 1996, Kulthe and Kothekar, 2011). It has been reported that up to 10 folds increases in the frequency of 5-methantryptophane resistant carrot cell increase up to 10 fold in presence of UV light (Kolb *et al*., 2001). The variation at the genetic level can be checked by adapting the DNA fingerprinting by using random markers (Piccioni *et al*., 1997). The present study was aimed to study the effect of sodium azide and UV mutation on callus cell lines and regeneration of *Daucus carota* plantlets and observe its effect through physiological estimation and to analyze mutation at the genetic level through DNA fingerprinting.

MATERIALS AND METHODS

The present study was conducted during the period from January 2012 to May 2012 at Genohelix Biolabs - A division of CASB, Jain University, Chamarajpet, Bangalore, Karnataka, India.

The chemicals which were used in the study are of "A.R. grade" from Nice, sd fine , Qualigens, Hi-media, Spectrochem, Colloids and Loba chemie.

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Daucus carota L. seeds were collected from Lal Bagh botanical garden to carry out the experiment. Collected seeds were taken in a conical flask and rinsed with tap water for 5-6 times and rinsed once with distilled water and surface sterilized using liquid detergent 2% (v/v) Savlon, 6-8 drops of Tween-20 for 15 min, rinsed with 70% ethanol for 30 sec, disinfected with 0.05% (w/v) $HgCl_2$ for 6 min and rinsed in sterile water several times to remove the traces of $HgCl_2$. The sterilized seeds were taken on a petri plate and were inoculated on 1X MS (Murashige and Skoog, 1962) media with 3% sucrose and 1% agar. In each bottle 5 seeds were inoculated for germination and plantlet development. The cultures were maintained in the culture room at a temperature of 25±2°C, light intensity of 1000 LUX, relative humidity between 50-60%, under photo-periodic regime for 16 h light and 8 h dark cycle. The seeds germinated and the plantlets were developed after 22 days of culture.

The *in vitro* grown plants of *Daucus carota* were taken on a petridish in the LAF and different parts of the plant were excised and inoculated on 1X MS media with 3% sucrose and 1% agar with different concentrations and combinations of growth hormones like 2,4-D and Kinetin. The cultures were incubated in the culture room under controlled conditions. Culture media inoculated with different *in vitro* grown plant parts showed faster and better response with callus formation on 1X MS basal medium with 3% sucrose fortified with 2 mg L^{-1} 2,4-D. The developed callus from *in vitro* grown plantlets were inoculated on 1X MS media with 3% sucrose fortified with 2 mg L^{-1} 2,4-D with different concentrations of sodium azide (200, 400, 600, 800 and 1000 μM) and were incubated for callus multiplication for 15 days.

Callus developed from *in vitro* grown plant were exposed to UV light for different time intervals (2-8 min) and inoculated on 1X MS media containing 3% sucrose fortified with 2 mg L^{-1} 2,4-D and incubated for callus multiplication for 15 days. After 15 days of culture mutated callus cell lines were inoculated on 1X MS media containing 3% sucrose fortified with 2 mg L^{-1} BAP for shoot induction and cultures were maintained under controlled culture conditions and data were collected.

Estimation of protein: One gram of developed plantlets was weighed, ground with 5 mL of 0.2 M phosphate buffer (pH 7) and filtered using Whatman's No. 1 filter paper. The filtrates were subjected to protein estimation by Lowry *et al.* (1951) with absorbance measured at 660 nm.

Estimation of total carbohydrate: One hundred milligram of developed plantlets was hydrolyzed with 2.5 N HCl by incubating in boiling water bath for 15 min and filtered using Whatman's No. 1 filter paper. The filtrates were estimated for total carbohydrate content by Anthrone method (Hedge and Hofreiter, 1962) with absorbance measured at 630 nm.

Table 1: List of RAPD Primers

Primers	Sequence 5'to3'
OPC-12	TGTCATCCCC
OPL-17	AGCCTGAGCC
OPW-03	GTCCGGAGTG
OPW19	CAAAGCGCTC
OPA-18	AGGTGACCGT
OPY-06	AAGGCTCACC
OPK-01	CATTCGAGCC
OPAB 19	ACACCGATGG
OPAB 7	GTAAACCGCC

Isolation of genomic DNA from Callus (CTAB DNA extraction): The *in vitro* grown plantlets from mutated callus cell lines from sodium azide and UV mutation were used for genomic DNA extraction by Cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). The DNA quality was assessed by examination on a 0.8% agarose gel stained with ethidium bromide.

RAPD amplification: RAPD amplification was performed in a volume of 25 μL that contained: 50 ng of template DNA, 2.5 mM each of dNTPs (Chromus Biotech Pvt. Ltd), 5 pM of primer (Sigma- Aldrich) (Table 1), 10x *Taq* assay buffer (10 mM Tris-HCl, pH -8.8, 500 mM KCl, 15 mM $MgCl_2$, 0.1% gelatin, 0.05% Tween 20 and 0.05% Nonidet P 40), 1 unit of *Taq* DNA polymerase (Chromus Biotech Pvt. Ltd) and sterilized water. Amplification was performed in a thermal cycler (MJ Research Inc.). Initial denaturation was performed at 94°C for 3 min before beginning the cycling protocol followed by 1 min at 92°C, 2 min at 36°C and 2 min at 72°C. A total of 40 cycles were performed. The cycling was terminated with a final extension at 72°C for 6 min. The amplification products were analyzed on 1.2% agarose gels with low range molecular weight marker. The gel was stained with ethidium bromide, visualized under ultraviolet light and documented using HEROLAB gel documentation system.

RESULTS AND DISCUSSION

Callus induction from stem and leaf explants of *in vitro* grown plantlets was observed on 1X MS medium with 3% sucrose, 1% agar fortified with 2 mg L^{-1} 2,4-D (Fig. 1a) when compared to other concentrations and combinations of 2,4-D and Kinetin. From all the different concentrations of sodium azide containing medium multiplication of callus was only achieved on 1xMS 3% sucrose, 1% agar fortified with 2 mg L^{-1} 2,4-D

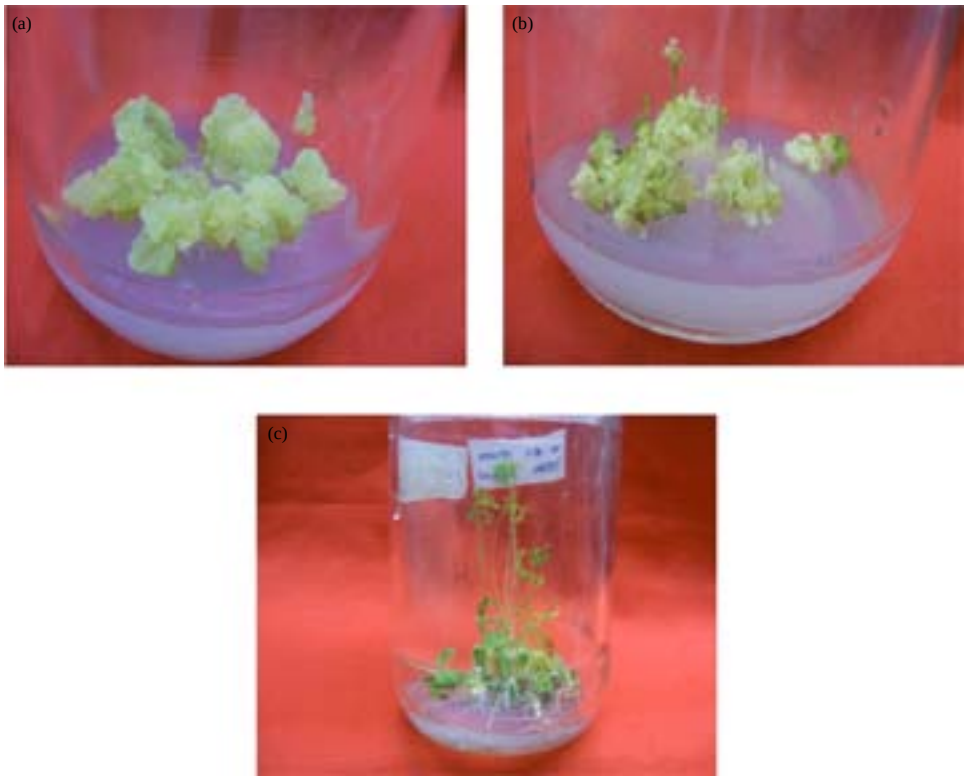

Fig. 1(a-c): (a) Well developed callus on 1X MS medium with 2 mg L^{-1} 2,4-D, (b) Shoot induction from the 4 min UV mutated callus cell line on 1X MS medium fortified with 2 mg L^{-1} BAP and (c) Well developed plantlet

containing 200 μM of sodium azide. Development of shoot and root from callus was achieved on 2 mg L^{-1} BAP containing medium after 24 day of incubation. The physiological estimation revealed that protein (0.160±0.04 mg mL^{-1}) and carbohydrate (0.720±0.06 mg mL^{-1}) contents in the cell lines (Fig. 2, 3). Callus exposed at different time intervals under UV radiation showed multiplication of callus in all the exposures. Development of shoot and root from callus was achieved on mg L^{-1} BAP containing medium after 24 day of incubation (Fig. 1b, c). The physiological estimation revealed that protein content (0.460±0.04 mg mL^{-1}) in 6 min exposed cell lines and carbohydrate content (0.820±0.08 mg mL^{-1}) in 4 min exposed cell lines which is high when compared to control cell lines and other sodium azide and UV exposure cell lines (Fig. 2, 3). Mutation was confirmed through DNA fingerprinting using RAPD markers. From all the 9 primers used OPK 01 gave best result with thick bands and amplification of the same when compared to other primers used, which shows strong sign of dominant expression of those bands which in turn alter the genetic character and stability, which shows the variation in banding pattern in mutated samples compared to control cell lines (Fig. 4).

Daucus carota L. is an important vegetable crop with high nutritional value. The changing climate, pollution, loss of vegetation, has affected the crop quality and there is a need to improve the variety. The present study was started with the aim to improve the cell lines through sodium azide and UV radiation induced mutation. In plant tissue culture, media plays a very important role for the regeneration. In the present investigation MS medium was used for the growth and development of *Daucus carota* based on the previous literatures (Pant and Manandhar, 2007; Tavares *et al*., 2010). Callus induction was well achieved on 2 mg L^{-1} 2, 4 D when compared to other concentrations and combinations of growth regulators which coincides with the earlier findings (Yau *et al*., 2008). Callus was exposed to UV radiation and sodium azide treatment with different time intervals and concentrations, respectively. Callus exposed to 6 min UV radiation has shown an increase in the protein content when compared to the other durations of exposure to the UV radiation. Sodium azide with 200 μM has shown no increase in protein content when compared to the normal cell lines. The concentration of sodium azide above 200 μM was lethal for the cell line which wont agrees with the previous

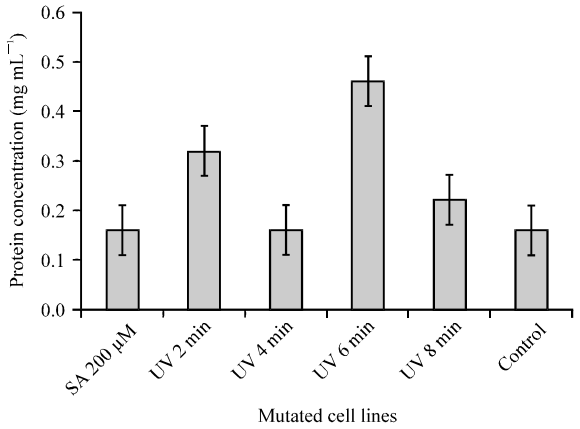

Fig. 2: Effect of sodium azide and UV mutation on Protein content in cell lines

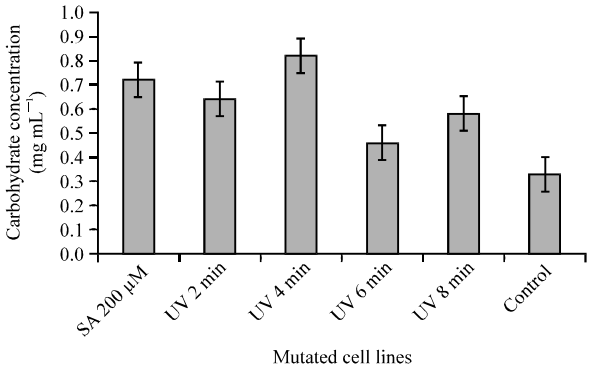

Fig. 3: Effect of sodium azide and UV mutation on carbohydrate content in cell lines

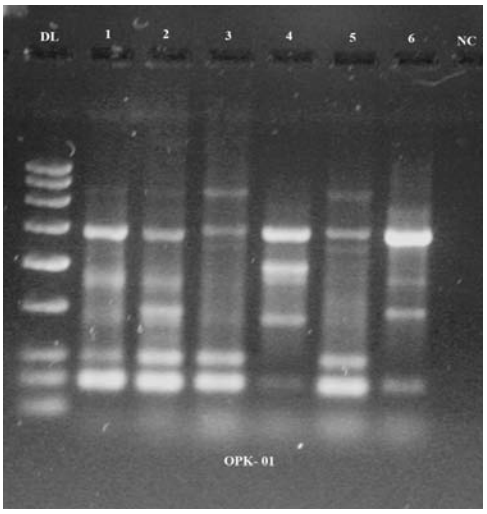

Fig. 4: RAPD amplification profile of mutated cell lines of *Daucus carota* L., 1: Control, 2: Sodium azide 200 µM, 3: UV 2 min, 4: UV 4 min, 5: UV 6 min, 6: UV 8 min, NC: Negative control

studies (Kulthe and Kothekar, 2011; Mostafa, 2011). Higher the concentration of sodium azide decrease in the callus growth was supported by the results of Khan *et al.* (2004, 2006), Sinha and Lai (2007) and Al-Gawwad and Makka (2009). Exposure to UV for 4 min and sodium azide with 200 µM has helped in increase in carbohydrate content when compared to the normal cell lines. UV radiation above 6 min has not supported the proper callus development which wont agrees with the findings of Ehsanpour and Razavizadeh (2005) in callus of *Medicago sativa*. The increase in protein and carbohydrate content has been supported with the DNA fingerprinting result with polymorphic banding pattern.

CONCLUSION

The results indicated that by sodium azide and UV mutation it was able to achieve the improvement of the cell lines of *Daucus carota* L. by positive mutation with the increase in the protein and carbohydrate content. Mutagenic treatments increase the genetic variability, which can be utilized for selection and improvement of the vegetable crops.

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REFERENCES

Akhaury, S.B., A.N. Sinha and A.K. Sinha, 1996. The effectiveness and efficiency of chemical mutagens on biological parameters in hull-less variety of *Hordeum vulgare* (L.) Karan 16. Neo Botanica, 4: 1-5.

Al-Gawwad, A. and A.H. Makka, 2009. Effect of chemical mutagens on *Mirabilis jalapa*. M.Sc. Thesis, Floriculture, Faculty of Agriculture, Alexandria University.

Arulbalachandran, D., L. Mullainathan and S. Velu, 2009. Screening of mutants in black gram (*Vigna mungo* (L.) Hepper) with effect of DES and COH in M_2 generation. J. Phytol., 1: 213-218.

Bertagne-Sagnard, B., G. Fouilloux and Y. Chupeau, 1996. Induced albina mutations as a tool for genetic analysis and cell biology in flax (*Linum usitatissimum*). J. Exp. Bot., 47: 189-194.

Broertjes, C. and A.M. van Harten, 1988. Applied Mutation Breeding for Vegetatively Propagated Crops. Elsevier, New York, USA., ISBN-13: 9780444427861, Pages: 345.

Bulk., R.W., H.J.M. Loffler, W.H. Lindhout and M. Koornneef, 1990. Somaclonal variation in tomato: Effect of explant source and a comparison with chemical mutagenesis. Theor. Applied Genet., 80: 817-825.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Ehsanpour, A.A. and R. Razavizadeh, 2005. Effect of UV-C radiation on drought tolerance of alfalfa (*Medicago sativa*) callus. Pak. J. Biol. Sci., 8: 1221-1224.

Hedge, J.E. and B.T. Hofreiter, 1962. Carbohydrates. In: Methods in Carbohydrate Chemistry, Whistler, R.L. and J.N. BeMiller (Eds.). Academic Press, New York, USA., pp: 1-19.

Khan, S., M.R Wani, M. Bhat and K. Parveen, 2004. Induction of morphological mutants in chickpea. Int. Chickpea Pigeonpea Newslett., 11: 6-7.

Khan, S., M.R. Wani and K. Parveen, 2006. Quantitative variability in mungbean induced by chemical mutagens. Legume Res., 29: 143-145.

Kolb, C.A., M.A. Kaser, J. Kopecky, G. Zota, M. Riedere and E.E. Pfundel, 2001. Effect of natural intensities of visible and ultraviolet radiation on epidermal UV screening and photosynthesis in grape leaves. Plant Physiol., 127: 863-875.

Kulthe, M.P. and V.S. Kothekar, 2011. Effects of sodium azide on yield parameters of chickpea (*Cicer arietinum* L.). J. Phytol., 3: 39-42.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randell, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mensah, J.K. and B. Obadoni, 2007. Effects of sodium azide on yield parameters of groundnut (*Arachis hypogaea* L.). Afr. J. Biotechnol., 6: 668-671.

Mostafa, G.G., 2011. Effect of sodium azide on the growth and variability induction in *Helianthus annuus* L. Int. J. Plant Breed. Genet., 5: 76-85.

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

Ojomo, A.O., O. Omueti, J.A. Raji and O. Omueti, 1979. Studies in induced mutation in cowpea, 5. The variation in protein content following ionizing radiation. Niger. J. Applied Sci., 21: 61-64.

Pant, B. and S. Manandhar, 2007. *In vitro* propagation of carrot (*Daucus carota*) L. Sci. World, 5: 51-53.

Piccioni, E., G. Barcaccia, M. Falcinelli and A. Standardi, 1997. Estimating alfalfa somaclonal variation in axillary branching propagation and indirect somatic embryogenesis by RAPD fingerprinting. J. Plant Sci., 158: 556-562.

Sinha, A. and J.P. Lai, 2007. Effect of mutagens on M_1 parameters and qualitative changes induced in M_2 generation in lentil. Legume Res., 30: 180-185.

Tavares, A.C., L.R. Salgueiro and J.M. Canhoto, 2010. *In vitro* propagation of the wild carrot *Daucus carota* L. subsp. halophilus (Brot.) A. Pujadas for conservation purposes. *In vitro* Cell. Dev. Biol. Plant, 46: 47-56.

Wongpiyasatid, A., S. Chotechuen, P. Hormchan, S. Ngampongsai and W. Promcham, 2000. Induced mutations in mungbean breeding: Regional yield trial of mungbean mutant lines. Kasetsart J. (Nat. Sci.), 34: 443-449.

Yau, Y.Y., S.J. Davis, A. Ipek and P.W. Simon, 2008. Early identification of stable transformation events by combined use of antibiotic selection and vital detection of Green Fluorescent Protein (GFP) in carrot (*Daucus carota* L.) callus. Agric. Sci. China, 7: 664-671.