Mycopath (2013) 11(2): 65-69 **Research Article**

In vitro selection of variants resistant to basal rot of garlic (*Allium sativum* L.) using induced mutations

[*]Zill-e-Huma Aftab and Saira Banarus

Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.
[*]Corresponding author's email: huma_mppl@yahoo.com

Abstract

Garlic (*Allium sativum* L.) variants resistant to basal rot disease were selected using induced mutations. Callus induction was achieved from basal portion on MS medium supplemented with 2, 4-D (3 mg L^{-1}). MS medium containing kinetin in combination with 2,4-D was optimized for callus proliferation. The calluses obtained were exposed to UV radiations for induction of mutation. The calluses showing minimum survival rate at 2.5 hours exposure were selected for pathogenecity test against culture filtrates of *Fusarium oxysporum* f. sp. *cepae*, the cause of basal rot of garlic. The calluses tolerant at 16% concentrations of culture filtrates were considered to be resistant.

Key words: Garlic; tissue culture; basal rot; disease resistance; mutagenesis.

Introduction

Garlic (*Allium sativum* L.) of the family Alliaceae is a bulbous perennial plant. It is widely cultivated crop due to its culinary and medicinal importance stemming from its biological activities including antibiotic, anticancer, antithrombotic and lipid lowering cardiovascular. The propagation rate of garlic in the field is very slow (approximately 5-10% per year) and takes many years to produce new verities for practical cultivation (Yan *et al*., 2009). Because all known cultivated verities of garlic are sterile, they can only be propagated vegetatively. This has prevented the production of new cultivars by plant breeding. An alternative method for creating new forms of a crop plant is by selecting soma-clonal variants from tissue cultured material.

Basal rot disease of garlic caused by *Fusarium oxysporum* f. sp. *cepae* is one of the most important world wide garlic diseases causing huge losses of crop usually with hidden symptoms in the field, (Schwartz and Mohan, 2006), but rotting of bulbs during storage. In vitro culturing of vegetatively propagated crops in combination with radiation-induced mutation has been proved to be a valuable method to produce variation and to rapidly multiply the mutants in disease free condition (Ayabe, 2001; Kamenetsky and Robinowitch, 2001; Fereol *et al*., 2002). In recent years, non specific and host specific phytotoxins have been recognized as a useful tool for the induction and selection of disease resistant clones in cell cultures. The present investigation was undertaken with the following objectives: development and identification of best protocol for in vitro regeneration of garlic, induction of mutation in garlic callus cultures against basal rot disease and selection of resistant callus against culture filtrates of *F. oxysporum* f. sp. *cepae*.

Materials and Methods

Surface sterilization:

Garlic bulbs of local variety (Desi), procured from local market of Lahore, Pakistan were broken in to individual cloves, peeled and treated with house hold detergent for 5 minutes followed by washing with tap water. Surface sterilization was done with with 0.1% $HgCl_2$ solution having 2 drops Tween 20 per 100 mL for 5 minutes followed by thorough washing with sterilized distilled water.

Callus Proliferation

Inner leaf segments excised from the sterilized cloves were cut into 2-3 mm long fragments and inoculated on MS (Murashige and Skoogs, 1962) medium supplemented with different concentrations of 2,4-D (0-4 mg L^{-1}) in combination with different concentrations of BAP (0-3 mgL^{-1}) and Kinetin (5-20mgL^{-1}). pH of the medium was adjusted at 5.5. Cultures were incubated at 26 $^{\circ}$C under both light (16 hours photoperiod from cool white light with an intensity of 2000-3000 Lux) and dark (24 hours dark period) conditions for comparison in callus proliferation rate. Periodic sub-culturing was done for continuous supply of nutrients.

Induction of mutation

Embryogenic and non embryogenic calluses were irradiated with UV radiations (from general

electric germicidal lamp emitting approximately 2600 A with an energy output of 25 ergs mm^{-2} S^{-1}) for 0, 0.5, 1.0, …..4.0 hours.

Isolation of fungus and preparation of culture filtrate

From infected garlic tissue *F. oxysporum* f. sp. *cepae* was isolated and maintained on 2% malt extract agar (MEA) medium. For preparation of aqueous culture filtrate, discs from fungal cultures were taken to inoculate 100 mL malt extract and incubated at 25 ^{o}C for 3 weeks on rotary shaker at 100 rpm. After 3 weeks mycelium and conidia were filtered through Whatman No. 1 filter paper, filtrate was used for screening of resistant calluses.

Screening of resistant calluses:

Pathogenecity test of the irriadated calluses for screening was performed by treating them with different concentrations (4-20% v/v) of culture filtrate of *F. oxysporum* f. sp. *cepae* in basic liquid MS medium.

Statistical analysis

Experiments were laid down in complete randomized block design with 3 replications. The data obtained from various parameters was analyzed using software package COSTAT.

Results and Discussion

Generally results revealed that 2, 4-D concentrations significantly effected the rate of callogenesis and the potential for producing embryos. Embryogenic calluses from basal portion under light condition were whitish green, soft and proliferating whereas non embryogenic calluses were yellowish in color. Percentage callus induction was the highest i.e. 80% after 10 days of incubation on MS medium containing 3 mgL^{-1} 2,4-D. Upper portion gave 60% callus induction after 17 days of incubation. The explants incubated under light condition showed 60% callus induction from basal portion of inner leaf segment after 10 days of inoculation. Both embryogenic and non embryogenic calluses were obtained (Al-Zahim *et al*., 1999; Robledo *et al*., 2000; Khan *et al*., 2004; Yan *et al*., 2009). There was a gradual decrease in callus induction above and below 3 mg L^{-1} 2,4-D concentration. The results are in line with the observations of Barandiaran *et al.* (1999).

MS medium supplemented with different concentrations of 6-benzyl amino purine (BAP) widely effected micropropagation of garlic from whole inner leaf segment. It is evident from the depicted results that micropropagation was 90% at 3 mg L^{-1} BAP. Medium without BAP did not show any response. It is worth noting that plant regeneration depends on diverse factors including the genotype, hormone supplements and so on.

For proliferation garlic callus were grown on different combined concentrations of kinetin and 2,4-D and the best results were exhibited at MS supplemented with10 mg L^{-1} Kinetin and 3 mg L^{-1} 2,4-D. Statistical analysis showed that significant variation was present among upper and basal portions, hormonal concentrations and dark and light period for callus development and proliferation.

Percentage survival of garlic calluses after exposure to UV light

For the induction of mutation, calluses were exposed to UV light for 0.5-4 hours and their survival rates were measured at each treatment after 14 days of irradiation. The survival rate reduced drastically after 2.5 hours treatment. Results depicted that calluses irradiated for 0.5, 1.0, 1.5, 2.0 and 2.5 hours gave 46.6, 36.3, 31.3, 23.3 and 6 % survival respectively. Exposure of more than 2.5 hours was lethal to the calluses. Similar results were also obtained by Baghwat and Duncan (1998), Chema *et al*. (2002) and Lee *et al*. (2003) on different plants. The worth of such variation has been documented in various papers aiming to develop or improve cultivars in almost all crops (Ahloowalia *et al*., 2004).

Screening of resistant calluses against culture filtrates of *Fusarium oxysporum*

For the screening of resistant calluses (exposed to UV rays for 2 and 2.5 hours) different concentrations of culture filtrates of *F. oxysporum* f. sp. *cepae* were used. The callus surviving in different concentrations were considered to be resistant (Gourd *et al*., 1998). The survival of the calluses of 2 hours exposure was 36.6% and 16.6% against 4% and 8% culture filtrate respectively. Results manifest that calluses exposed for 2.5 hours to the UV rays were more resistant to the culture filtrate as the achieved survival rate was 40% at 4% concentration of the culture filtrate.

It has been shown previously that phytotoxicity of crude culture filtrates from *F. subglutinans* was higher on culture of pineapple cultivars that were more susceptible to the disease than those from the resistant (Borras *et al*., 1998). The present results support this observation that culture filtrate of *F. oxysporum* f. sp. *cepae* applies at various concentrations reduced the survival rate

of garlic calluses and the effect was correlated with the resistance of calluses exposed to UV rays for 2.5 hours. Same symmetry of the results was observed by Ahmad *et al*. (1991); Jin *et al*. (1998) and Gonzalez *et al*. (2006). It is optimistically assumed by looking at the results obtained so far and the work that is continuing that garlic variants resistant to basal rot caused by *F. oxysporum* f. sp. *cepae* can be produced by the use of *in vitro* selection technology combined with induced mutations (Maluzynski *et al*., 2000). Further work on these parameters is in progress before evidence might be given on this subject matter.

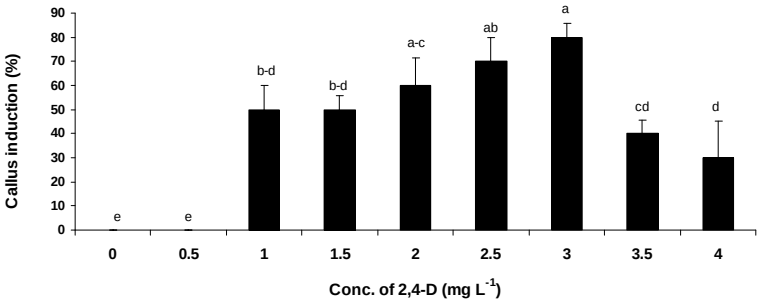

Fig. 1: Effect of different concentrations of 2,4-D on callus induction from basal portion of inner leaf segment.

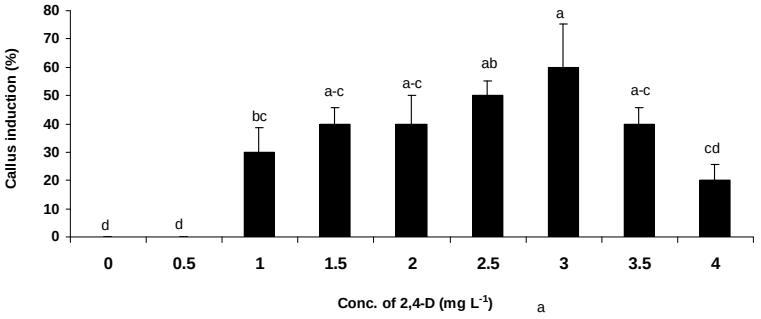

Fig. 2: Effect of different concentrations of 2,4-D on callus induction from upper portion of inner leaf segment.

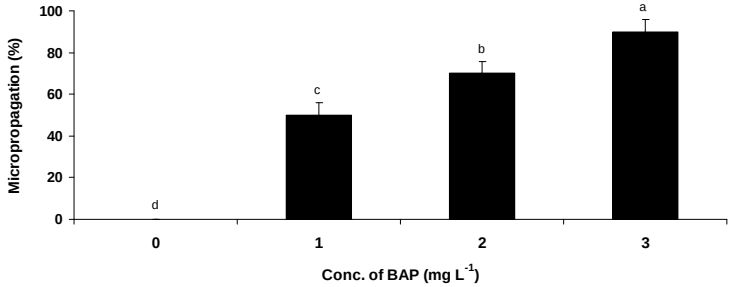

Fig. 3: Effect of different concentration of BAP on micropropagation of garlic.

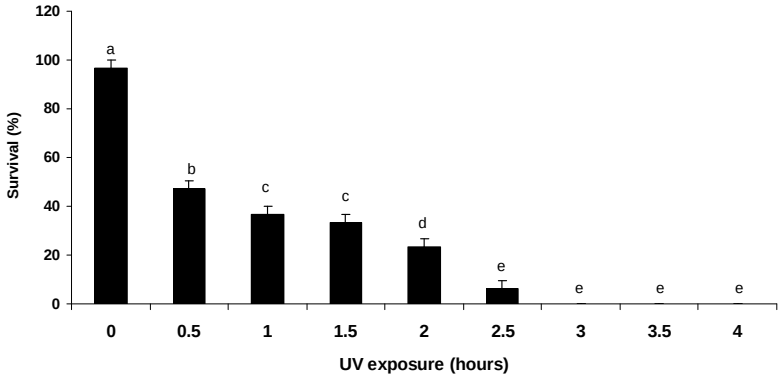

Fig. 4: Effect of UV mutation on percentage survival of garlic calluses.

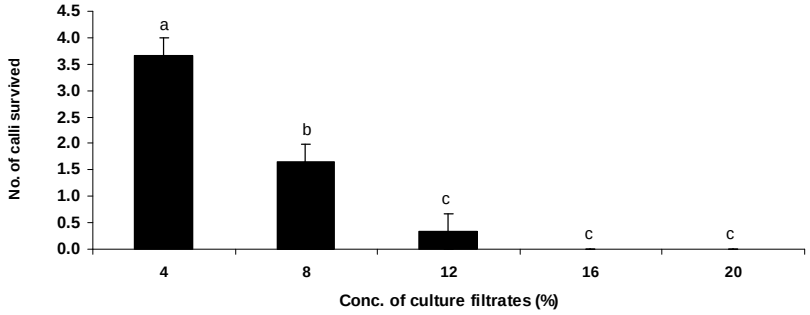

Fig. 5: Effect of culture filtrates of *F. oxysporum* on garlic calluses exposed to UV for 2.0 hours.

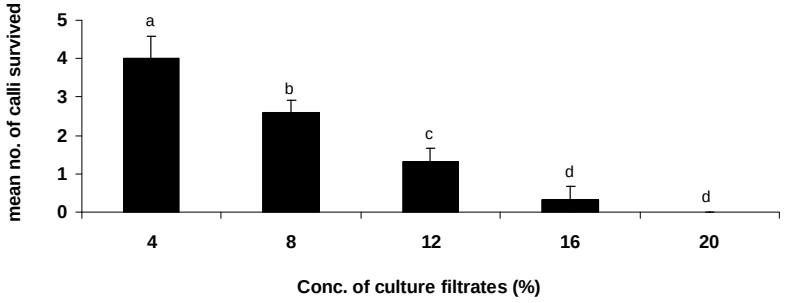

Fig. 6: Effect of culture filtrates of *F. oxysporum* on garlic calluses exposed to UV for 2.5 hours.

References

Ahloowalia BS, Malusznyski M, Nichterlin K, 2004. Global impact of mutation derived varieties. *Euphytica*, **135**: 187-204.

Ahmad I, Day JP, MacDonald MV, Ingram GS, 1991. Haploid culture and UV mutagenesis in rapid-cycling Brassica napus for the generation of resistance to chlorsulfuron and *Alternaria brassicicola*. *Ann. Bot.*, **67**: 521-525.

Al-Zahim MA, Ford Lloy BV, Newbury HJ, 1999. Detection of somaclonal variation in Garlic (Allium sativum L.) using RAPD and cytological analysis. *Plant Cell Rep*., **18**: 473-477.

Ayabe M, 2001. A novel and efficient tissue culture method "stem –disc dome culture" for producing virus free garlic (*Allium sativum* L.). *Plant cell Rep*., **20**: 503-507.

Barandiaran X, Martin N, Rodriguez Conde MF, Pietro AD, Martin J, 1999. An efficient method for callus cultures and shoot regeneration of garlic (*Allium sativum* L.). *Hort. Sci.*, **34**: 503-507.

Bhagwat B, Duncan EJ, 1998. Mutation breeding of highgate *Musa acuminata* group for tolerance to *Fusarium oxysporum* f. sp. *cubense* using gamma radiation. *Euphytica*, **101**: 143-150.

Borras O, Santos R, Matos AP, Cabral RS, Tapia R, Arizola M, Prez MC, 1998. Phytotoxic effect of culture filtrate from *Fusarium subglutinans*, the causal agent of fusariose of pineapple (*Ananas comosus* L.). *Euphytica*, **19**: 74-76.

Cheema AA, Saleem MY, Awan MA, 2002. In vitro techniques for the selection of basmati rice mutatnts batter adapted to saline environments In: Mutation, in vitro and Molecular Techniques for Environmentally Sustainable Crop Improvement. Mauszynski M and Kasha KJ (eds.). IAEA, Vienna. pp. 161-168.

Fereol L, ChovelonV, Causse S, Michaux-ferriere N, Kahane R, 2002. Evidence of a somatic embryogenesis process for plant regeneration in garlic (*Allium sativum* L.). *Plant Cell Rep*., **21**: 197-203.

Gonzalez AI, Polanco C, Ruiz ML, 2006. In vitro culture response of common bean explants to filtrate from *Pseudomonas syringae* pv. *phaseolicola* and correlation with disease resistance. *In vitro Cell Dev. Plant Biol.*, **42**: 160-164.

Gourd JM, Southward GM, Philips G, 1998. Response of *Allium* tissue cultures to filtrates of *Pyrenochaete terrestris*. *Hort. Sci.*, **23**: 776-778.

Jin H, Hartman GL, Nickell D, Widholm JM, 1996. Phytotoxicity of culture filtrate of *Fusarium solani*, the causal agent of sudden death syndrome of soybean. *Plant Dis.*, **80**: 922-927.

Kamenetsky R, Robinowitch HD, 2001. Floral development in bolting garlic. *Sex Plant Rep*., **13**: 235-241.

Khan N, Alam MS, Nath UK, 2004. *In vitro* regeneration of garlic through callus cultures. *J. Biol. Sci*., **4**: 189-191.

Lee IS, Kim DS, Hyun DY, Lee SJ, Song HS, Lim YP, Lee YI, 2003. Isolation of gamma-induced rice mutants with increased tolerance to salt by anther culture. *J. Plant Biotechnol.*, **5**: 51-57.

Maluszynski M, Nichterlein K, Van Zanten L, Ahloowalia BS, 2000. Officially released mutant varieties-the FAO/IAEA database. Mutation breeding Review No. 12 December. Joint FAO/IEAE Vienna, Austria.

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

Robledo Paz A, Villalobos Arambula VM, Jofre Garfias AE, 2000. Efficient plant regeneration of garlic (Allium sativum L.) by root tip cultures. *In Vitro Cell Dev. Biol. Plant*, **36**: 416-419.

Schwartz HF, Mohan K, 2006. Compendium of onion and garlic diseases and pests, 2nd Ed, APS Press.

Yan MM, Xu C, Kim CH, Um YC, Guo DP, 2009. Effects of explant type, culture media and growth regulators on callus induction and plant regeneration of Chinese jiaotou (*Allium chinense*). *Sci. Hort.*, **123**: 124-128.