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Review Article
Fungal Toxin as Potential Tool for *in vitro* Selection and Regeneration of Resistant Plants

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Abstract
Fungal pathogens impose a foremost threat to crop productivity. So, efforts to develop resistance against these pathogens in plants are of great importance. In recent years *in vitro* selection has emerged as a viable tool for developing resistant germ plasm. Resistant variants can be acquired by applying selective agent such as fungal toxins in the tissue culture media. In toxin based *in vitro* selection, explants that can withstand selection pressure can survive and only those cells are selected for regeneration. The resistance trait appearing in the regenerated plants may be genetically stable and useful in the crop improvement. Henceforth, the present review focuses on implementation of fungal toxin as a potential tool for *in vitro* selection and recent progress made towards the development of resistant germ plasm through this technique.

Key words: Callus, double layer, fungal culture filtrate, gradient system, *in vitro*

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INTRODUCTION

The production and yield of majority of crops are severely influenced by various diseases caused by necrotrophic phytopathogen[1]. Damages caused by these pathogens results into huge economic losses at global level. Conventional breeding strategies had always played an important role in crop improvement. Though, this traditional method has gained less success and has failed to some extent to generate the desired outcome. Henceforth, it had turned to be essential to set up biotechnological methods for solving such vital problem regarding crop improvement.

Among the biotechnological techniques, (transgenic technique[2], elicitors mediated plant defense etc[3-5]), *in vitro* culture has turned to be an imperative means to study host pathogen interaction not only at the cellular but also at molecular level[6] in a small space under controlled environment condition, which are nearly impossible to regulate with conventional system. Infact plant cells under *in vitro* culture, respond to various stresses in a pattern analogous to that of an intact plants. This facilitates *in vitro* cell culture as an ideal tool for understanding the resistance related responses and dynamic changes taking place both at the cellular and subcellular level upon infection with the pathogenic microorganism[7].

Pathogenic microbes including fungi produces a wide range of toxins that may either have adverse or stimulatory impact in plant system[8]. An interesting characteristic of many of those toxins are their capacity to stimulate several resistance related responses in the host[9] including (1) Alteration in primary[10] and secondary metabolites[11], (2) Accumulation of defense related components[9,12,13], (3) Hypersensitive responses[14] or (4) Resulting to systemic acquired resistance (SAR) and induced systemic resistance (ISR)[15]. This attribute had encouraged several researchers to utilize fungal toxin as a selective agent for obtaining resistant germ plasm through *in vitro* approach.

The *in vitro* culture in conjunction with selective agent offers prospect to isolate cells with desirable trait. Among the selective agents, toxins derived from necrotrophic fungal pathogens have received more consideration because of the simplicity in exposing cells to a controlled and sublethal dosage of isolated toxin[16]. The sublethal dose of toxin when applied in cell culture media, develops a selection pressure that allows only the resistant cells to survive or divide. These resistant cells can then be subjected to regeneration to obtain the whole plant. On regeneration, in majority of cases the *in vitro* selected tolerant cells show significant degree of resistance or tolerance not only in response to toxin but also against the pathogen[17]. Besides all these an importance of this selection method lies on the fact that it significantly shortens the time duration for the selection of desirable character under selection pressure with negligible environmental interference, thereby complementing field selection[18]. These attributes constitute toxin based *in vitro* selection strategy a feasible, non-controversial biotechnological means for developing resistant varieties. The present paper summarized the recent knowledge about fungal toxin as a selective agent and its potential application in *in vitro* selection for inducing resistance against diseases.

Fungal toxin as selective agent: Selection of precise selective agent is the most vital step for establishing a screening system[19]. In recent years application of pathogen mainly fungal toxin for *in vitro* selection are more encouraged by researchers. Exploiting toxins for *in vitro* culture is found to be more in advantageous in comparison to pathogen because cells can be exposed easily and evenly to toxin via dispersing the *in vitro* cell in toxin solution or by plating them on toxin supplemented media[20].

The basic parameter that must be satisfied prior a toxin is implemented as a selective agent are, primarily the toxin produced by the pathogen must be involved with the development of disease. Secondly the toxin should directly act at the cellular level[19,21]. Lastly one or more compound present in the selective agent must also occur in the infected tissue of the host plant[22].

Fungal toxin includes a range of secondary metabolites such as low molecular weight peptides, terpenoides and carbohydrates[23,24]. Based on the specificity toxins are divided into host specific (HST) and non -host specific (nHST) toxin. HSTs affect only a selective plant species. These toxins are produced by various pathotypes of the fungal pathogens such as AAL- , ACT-, AK-, AM-, AF-[25,26], Victorin[27], HC toxin[28], T-toxin[29] etc.

Non-specific toxins (nHSTs) exhibit activity at broader range affecting several plants irrespective of whether they are host or non-host of the pathogen[26,30]. Activities of this toxin are not restricted to phylogenetic specialization and generally rely on concentration[24]. Few examples of nHSTs are Zinniols, tenuazonic acid[26], Brefeldin A (dehydrogenase), curvularin and tentoxin[31,32] fusaric acid[33,34], fusicoccin[8] etc. These nHST exercise their effect by various modes in plants.

Among the above mentioned toxins few toxins was applied as a selective agent for *in vitro* selection experiments detailed[35-53] in Table 1.

Table 1: List of fungal toxins used as selective agent for *in vitro* selection of plant

Fungal toxin	Plants	References
Colletotrichum gloeosporioides toxin	Mango	Jayasankar *et al.*[35]
Colletotrichum falcatum phytotoxin	Sugarcane	Mohanraj *et al.*[36]
Fusaric acid	Banana	Matsumoto *et al.*[37]
Fusaric acid (*Fusarium subglutinans*)	Pineapple	Borras *et al.*[38]
Fusaric acid	Barley	Chawla and Wenzel[39]
Fusaric acid	*Gladiolus*	Remotti *et al.*[40]
Fusarium oxysporum (Fao) toxin	Date palm	El Hadrami *et al.*[41]
Fusaric acid	Alfalfa	Nedelnik and Repkova[42]
Fusaric acid	Tomato	Shahin and Spivey[43]
Alternaria alternata toxin	Sunflower	Kintzios *et al.*[44]
AT toxin (*Alternaria alternata*)	Tobacco	Ishida and Kumarshiro,[45]
AAL toxin (*Alternaria alternata*)	Tomato	Kodama *et al.*,[46]
AM toxin (*Alternaria alternata*)	Apple	Saito *et al.*[47]
Victorin (*Helminthosporium victoriae*)	Oat	Rines and Luke,[48]
HC toxin (*H. carbonum*)	Corn	Wenzel and Foroughi-Wehr[49]
Toxic extract of *Septoria nodorum*	Wheat	Keller *et al.*[50]
Drechslera teres toxin	Barley	Hunold *et al.*[51]
Oxalic acid *(Sclerotium cepivorum)*	*Allium*	Sayed *et al.*[52]
Cercosporin	Mung bean	Kumar *et al.*[53]

***In vitro* selection techniques:** For *in vitro* selection using fungal toxin as a selective agent several methods are developed. The details of various techniques are discussed below.

Double layer culture method: In this strategy, fungal culture that forms the first layer is overlaid with culture growth medium supplemented with fungicides mycostatin or benlate at fungistatic concentration. Following diffusion of the toxic metabolite from the fungal layer, calli are placed on the upper layer of the medium[54]. However, this method was further modified by Ahmed *et al.*[55], for *in vitro* selection of wheat calli to obtain resistant lines to *Fusarium* toxin. In the modified method the fungal culture was autoclaved to kill the fungal cells. This formed the first layer. This layer was overlaid with the callus growth medium forming the second layer. The calli are then placed on the second layer following diffusion of toxic material from the first layer to the callus growth medium[55]. Later, Kleijer *et al.*[56] used this method for *in vitro* selection of Triticale for inducing resistance to head blight disease caused by *Fusarium* sp. This method was also used for selection of embryogenic calli of carnation cultivars for resistance to toxic metabolites of *Fusarium oxysporum* by Esmaiel *et al.*[57].

Partitioned culture technique: In this method the adjacent quadrants of the petri dish was used for culture. After mycelial growth, walls between the sectors were pierced for diffusion of the fungal toxic metabolites. Following this, the medium supplemented with fungicides were then poured in the compartments where callus are to be transferred[54]. Resistant calli were then selected and transferred to toxin free medium for further growth and regeneration.

Gradient system: In gradient method, cultures are exposed to culture media with a gradual increase in concentration of selection agent. Through this system, a sublethal dosage of toxin can be optimized that can further be used for *in vitro* selection experiment. Gradient system for *in vitro* selection had been applied in several plants. For instance, Kintzios *et al.*[44] studied *in vitro* reaction in sunflower by allowing the growth of callus in selection media amended with gradient of *Alternaria alternata* toxin. Similarly a gradual increase in fusaric acid concentration to cell suspension culture of *Gladiolus* decreased the cell growth. However, at this sublethal dose plantlets of the selected cell lines were showed significant resistance over that of control[58]. Gradient method was also applied by Chopra *et al.*[59] for selection of resistance in *Brassica* using *Alternaria brassicae* toxin.

Continuous-discontinuous selection method: This technique was adapted by Tripathi *et al.*[60] for *in vitro* selection of resistant cell lines of onion using purified toxic culture filtrate of *Alternaria porri*. In this strategy two selection method were used -a continuous method, where four cycles of selection were performed on toxic media (continuous method) and a discontinuous system in which a pause was given after second and third cycle of selection using non-toxic medium. The continuous and discontinuous method proved to be superior as it allowed the callus to regain their regeneration potency[60].

Direct selection: This method involves direct exposure of *in vitro* cells to the selective media amended with toxin. Direct

selection proved to give better results in several plants. As for instance, in barley 8-11% resistant calli were obtained when grown in a media supplemented with 0.8 mM fusaric acid after four cycle of selection[39]. Arcioni *et al*.,[61] also implemented this method for *in vitro* selection of alfalfa calli. Srinath and Padmaja[62], for obtaining resistant lines in sunflower, directly exposed callus of sunflower to 40% *Alternaria* culture filtrate (ACF) supplemented in the culture media.

Biochemical characterization of disease resistance during *in vitro* selection: When *in vitro* plant cells are subjected to selective pressure, several defense related molecules induced that lead to resistance in the *in vitro* tissue[63]. The tolerance in these calli or cells can be studied by analysis of various biochemical alterations including oxidative stress, activation of defense enzymes and secondary metabolites. The oxidative damage induced by fungal toxic metabolites leads to activation and deactivation of various antioxidant enzymes that play a considerable role in defense mechanism[64,65]. The antioxidant enzymes include peroxidase (POD), ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR)[64]. Besides these, enzymes like β-1, 3-Glucanase and PAL are also induced in response to toxin in calli under *in vitro* culture[9]. For instance, Singh *et al*.[66] reported an increase in peroxidase and β-1,3-Glucanase activities in calli of *in vitro* selected resistant cell line of chickpea. Kumar *et al*.[65] examined antioxidant enzymes in fungal culture filtrate of *Alternaria carthami* in tolerant plant of safflower cv.NARI. Among enzymes, an increase in POD and SOD activity and a slight decrease in CAT level were observed in the FCF (Fungal culture filtrate) tolerant plants. An increase in PAL, peroxidase and phenolic acid in the *in vitro* selected calli of *Medicago sativa* resistant to *Fusarium* phytotoxin was observed by Hrubcova *et al*.[67].

Henceforth disease resistance during *in vitro* selection can be characterized through increased expression of PR proteins, antifungal compounds and production of phytoalexin in plants[65,68]. Still only those *in vitro* cells exhibiting quick activation of defense mechanism in a sustained way stay viable and survive during recurrent procedure of *in vitro* selection[63]. The plants that are regenerated from these *in vitro* cells may comprise increased level of defense genes expression in a constitutive way making plants more resistant in comparison to the parent[63].

Application of *in vitro* selection for crop improvement: Use of fungal toxin as selection agent for screening *in vitro* cultures is now well established. In the past few decades most of the researches based on *in vitro* selection strategy for crop improvement are published. Some examples of the application of toxin-based *in vitro* selection for crop improvement are summarized as follows.

For instance, in maize, resistance was induced using T-toxin produced by *Helminthosporium maydis*. The resultant regenerates showed resistance to pathogen[69]. Sacristan[70], applied toxins as a selection agent and screened resistance in *Brassica napus* to fungal pathogen *Phoma lingam*. Behnke[71] selected *Solanum tuberosum* calli tolerant to the toxic filtrate of *Phytophthora infestans*. The resultant calli showed resistance to all four pathotypes of the pathogen. Tobacco callus expressed resistance to toxin from *Alternaria alternata* (leaf spot disease). The plants regenerated from these calli exhibited resistance to the diseases[72]. Chawla and Wenzel[39] using purified culture filtrate (PCF) produced by *Helminthosporium sativum*, screened callus of barley and wheat for their resistance. The regenerants from those callus lines survived toxin treatment and *in vivo* testing against pathogen, revealing majority of plants were less sensitive. Gentile *et al*.[73] regenerated lemon resistant to the disease "mal secco" caused by *Phoma tracheiphila* by screening cell lines of nucellar origin using partially purified toxin produced by the fungal pathogen. Remotti *et al*.[40] effectively selected cell lines of *Gladiolus*× *Grandiflorus* cv. *Peter* and regenerated plants resistant to toxin-fusaric acid produced by the fungal pathogen *Fusarium oxysporum*. Nyange *et al*.[74] established the selective impact of filtrate of fungus *Colletotrichum kahawae* on *in vitro* culture of cell and protoplast of *Coffea arabica*. Yang *et al*.[75] selected *F. graminearum* tolerant plantets of *Triticum aestivum* by successfully *in vitro* screening using deoxynivalenol as selection agent. Srinath and Padmaja[62], obtained plantlets of chickpea (*Cicer arietinum*) from selected calli resistant to culture filtrate of *F. oxysporum* showed resistance to wilt. Jayasankar *et al*.[7] demonstrated that the selected lines of grapevines in response to culture filtrate of *Elsinoe ampelina* inhibited growth of *E. ampelina* and *Fusarium oxysporum*, expressing resistance in the whole plant. Thakur *et al*.[76], applied *in vitro* strategy for inducing resistance in two cultivars of *Dianthus caryophyllus* resulting from callus cultures by applying toxic culture filtrate of *F. oxysporum*. Nearly 32% of the regenerated plants derived from resistant callus exhibited significant resistance in response to pathogen in field, showing no variation in phenotype in the selected regenerants. Gayatri and Kavyashree[77] obtained clones tolerant to root rot disease in turmeric variety Suguna (*Curcuma longa*) against pure culture filtrate (PCF) of the pathogen *Pythium graminicola* by method of *in vitro* selection. The disease tolerant clones showed 90% survival

frequency. Regenerants of *Pelargonium graveolens* (a rose scented geranium) obtained from callus resistant to culture filtrate of *Alternaria alternata*, showed induced disease resistance in geranium plant at the cellular level[16]. Valencia *et al.*[78], obtained calli of pineapple treated with various concentrations of *Phytophthora cinnamomi* crude culture filtrate. Calli resistant to the crude filtrate of pathogen *P. cinnamomi* were selected followed by regeneration into plantlets subsequent to three selection cycles. Nurcahyani *et al.*[79] applied fusaric acid as selective agent to obtain vanilla plantlets resistant to *Fusarium oxysporum*. Oxalic acid, a phytotoxin produced by *Sclerotium cepivorum* was used by Sayed *et al.*[52] for *in vitro* selection of onion for inducing disease resistance against white rot disease.

Constraints in toxin based *in vitro* selection: *In vitro* approach represents a viable integration of traditional breeding method for crop improvement, specifically against necrotrophic phytopathogens. Though several instances are available on *in vitro* selection for disease resistance but only a few somaclones are declared successful as varieties.

Another major constraints encountered in this method was the reduction or loss of regeneration ability of selected cell lines upon exposure to toxin for a longer period of time. One reason behind this constraint can be cytogenetic alternation that is induced under selection pressure of *in vitro* cells[80]. This problem was reported by Nasir and Riazuddin[58] in cell lines of *Gladiolus* upon exposure to fusaric acid for longer duration.

Penetration and transportation of the toxic media into callus is another difficulty. This was resolved by Saxena *et al.*[16] in *Pelargonium* sp. by allowing the calli to grow in liquid media facilitating maximum transportation of the toxin into the cells.

CONCLUSION AND FUTURE PROSPECTS

For the last few decades the incredible progress made in the field of plant biotechnology for generating resistant genotype is an indication of great enthusiasm in the scientific community on this issue. Fungal toxin based *in vitro* selection offers a significant means for studying various physiological, biochemical and molecular mechanism in plants. In recent years, extensive advancement has been made concerning the development and isolation of resistant genotypes by using *in vitro* techniques. *In vitro* selection is a feasible approach as it save time that is actually required for developing a disease resistant line of commercially important crops. Assessment of these resistant plants obtained by *in vitro* selection method further need to be evaluated at field level and proper agronomic data should be obtained to certify that this technique can be effectively applied in fields to boost the disease management practices. Apart these a significant feature which make this biotechnological approach more important is that the selection of resistant variants is neither protected under intellectual property regulations, nor it is matter of public safety concerns for development of novel crop cultivars. Thus this method of selection is becoming a promising, non transgenic approach which offers an attractive method for producing improved cultivars. Henceforth, in future toxin based *in vitro* selection technique in concert with molecular approaches and functional genomics can turn to be a better option that will offer a novel prospect to develop disease resistant plants.

SIGNIFICANCE STATEMENT

The review systematically explains the potential role of fungal toxin in *in vitro* selection and regeneration of resistant germ plasm. This article will help the researchers to uncover the critical areas of inducing resistance in the *in vitro* tissue using toxin as a selective agent that many researchers would become enthusiastic to explore this area further. Thus a new avenue might be explored scientifically to develop many new resistant plants.

REFERENCES

1. Laluk, K. and T. Mengiste, 2010. Necrotroph attack on plants: Wanton destruction or convert extortion? Arabidopsis, Vol. 8. 10.1199/tab.0136.
2. Tohidfar, M. and S. Khosravi, 2015. Transgenic crops with an improved resistance to biotic stresses: A review. Biotechnol. Agron. Soc. Environ., 19: 62-70.
3. Chandra, S., N. Chakraborty, A. Dasgupta, J. Sarkar, K. Panda and K. Acharya, 2015. Chitosan nanoparticles: A positive modulator of innate immune responses in plants. Sci. Rep., Vol. 5. 10.1038/srep15195.
4. Chandra, S., N. Chakraborty, K. Panda and K. Acharya, 2017. Chitosan-induced immunity in *Camellia sinensis* (L.) O. Kuntze against blister blight disease is mediated by nitric-oxide. Plant Physiol. Biochem., 115: 298-307.
5. Chakraborty, N., S. Chandra and K. Acharya, 2017. Biochemical basis of improvement of defense in tomato plant against *Fusarium* wilt by $CaCl_2$. Physiol. Mol. Biol. Plants, 23: 581-596.
6. Jayasankar, S. and D.J. Gray, 2004. *In vitro* Plant Pathology. In: Plant Development and Biotechnology, Gray, D.J. and R.N. Trigiano (Eds.)., CRC Press, New York, pp: 293-299.
7. Jayasankar, S., Z. Li and D.J. Gray, 2000. *In-vitro* selection of *Vitis vinifera* chardonnay with *Elsinoe ampelina* culture filtrate is accompanied by fungal resistance and enhanced secretion of chitinase. Planta, 211: 200-208.

8. Pusztahelyi, T., I.J. Holb and I. Pocsi, 2015. Secondary metabolites in fungus-plant interactions. Front. Plant Sci., Vol. 6. 10.3389/fpls.2015.00573.
9. Gupta, N.S., M. Banerjee, S.K. Basu and K. Acharya, 2013. Involvement of nitric oxide signal in '*Alternaria alternata*' toxin induced defense response in '*Rauvolfia serpentina*' Benth. ex Kurz calli. Plant Omics J., 6: 157-164.
10. Rojas, C.M., M. Senthil-Kumar, V. Tzin and K. Mysore, 2014. Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Front. Plant Sci., Vol. 5. 10.3389/fpls.2014.00017.
11. Wojakowska, A., D. Muth, D. Narozna, C. Madrzak, M. Stobiecki and P. Kachlicki, 2013. Changes of phenolic secondary metabolite profiles in the reaction of narrow leaf lupin (*Lupinus angustifolius*) plants to infections with *Colletotrichum lupine* fungus or treatment with its toxin. Metabolomics, 9: 575-589.
12. Gupta, N.S. and K. Acharya, 2015. A fungal toxin elicit nitric oxide signal mediated innate immune response in *Rauvolfia*. Plant Cell. Biotechnol. Mol. Biol., 16: 171-180.
13. Gupta, N.S. and K. Acharya, 2016. *Alternaria alternata* toxin mediated elicitation of secondary metabolite pathway in *Rauvolfia serpentina* along with disease protection. Der Pharm. Lett., 8: 146-153.
14. Kombrink, E. and E. Schmelzer, 2001. The hypersensitive response and its role in local and systemic disease resistance. Eur. J. Plant Pathol., 107: 69-78.
15. Cipollini, D., S. Enright, M.B. Traw and J. Bergelson, 2004. Salicylic acid inhibits jasmonic acid induced resistance of Arabidopsis thaliana to *Spodoptera exigua*. Mol. Ecol., 13: 1643-1653.
16. Saxena, G., P.C. Verma, L.U. Rahman, S. Banerjee, R.S. Shukla and S. Kumar, 2008. Selection of leaf blight-resistant *Pelargonium graveolens* plants regenerated from callus resistant to a culture filtrate of *Alternaria alternate*. Crop Prot., 27: 558-265.
17. Slavov, S., 2005. Phytotoxins and *in vitro* screening for improved disease resistant plants. Biotechnol. Biotechnol. Eq., 19: 48-55.
18. Jain, S.M., 2001. Tissue culture-derived variation in crops. Euphytica, 118: 153-166.
19. Chandra, R., M. Kamle, A. Bajpai, M. Muthukumar and S. Kalim, 2010. *In vitro* selection: A candidate approach for disease resistance breeding in fruit crops. Asian J. Plant Sci., 9: 437-446.
20. Daub, M.E., 1986. Tissue culture and the selection of resistance to pathogens. Annu. Rev. Phytopathol., 24: 159-186.
21. Hammerschlag, F.A., 1984. *In vitro* Approaches to Disease Resistance. In: Advances in Agricultural Biotechnology: Application of Genetic Engineering to Crop Improvement, Collins, G.B. and J.G. Petolino (Eds.). Martinus Nijhoff/Dr W. Junk Publishers, Netherlands, pp: 453-489.
22. Lebeda, A. and L. Svabova, 2010. *In vitro* Screening Methods for Assessing Plant Disease Resistance. In: Mass Screening Techniques for Selecting Crops Resistant to Disease, IAEA (Ed.)., IAEA, Vienna, Austria, pp: 5-45.
23. Huang, J.S., 2013. Plant Pathogenesis and Resistance: Biochemistry and Physiology of Plant-Microbe Interactions. Springer Science and Business Media, The Netherlands, Pages: 692.
24. Berestetskiy, A.O., 2008. A review of fungal phytotoxins: From basic studies to practical use. Applied Biochem. Microl., Vol. 44. 10.1134/S0003683808050013.
25. Tsuge, T., Y. Harimoto, K. Akimitsu, K. Ohtani and M. Kodama *et al*., 2012. Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternate*. FEMS Microbiol. Rev., 37: 44-66.
26. Meena, M., S.K. Gupta, P. Swapnil, A. Zehra, M.K. Dubey and R.S. Upadhyay, 2017. *Alternaria* toxins: Potential virulence factors and genes related to pathogenesis. Front. Microbiol., Vol. 8. 10.3389/fmicb.2017.01451.
27. Akpaninyang, F.E. and E.U. Opara, 2017. The influence of toxins in disease symptom initiation in plants: A review. J. Agric. Sustain., 10: 29-52.
28. Walton, J.D., 2006. HC-toxin. Phytochemistry, 67: 1406-1413.
29. Turgeon, B.G. and S.E. Baker, 2007. Genetic and genomic dissection of the *Cochliobolus heterostrophus* Tox1 locus controlling biosynthesis of the polyketide virulence factor T toxin. Adv. Genet., 57: 219-261.
30. Mamgain, A., R. Roychowdhury and J. Tah, 2013. *Alternaria* pathogenicity and its strategic controls. Res. J. Biol., 1: 1-9.
31. Thomma, B.P.H.J., 2003. *Alternaria* spp.: From general saprophyte to specific parasite. Mol. Plant Pathol., 4: 225-236.
32. Saharan, G.S., N. Mehta and P.D. Meena, 2016. Resistance. In: Diseases Of Crucifers: Biology, Ecology and Disease Management, Saharan, S., Govind, Mehta, Naresh, Meena and P. Dayal (Eds.)., Springer, Singapore, pp: 175-210.
33. Li, C., C. Zuo, G. Deng, R. Kuang and Q. Yang *et al*., 2013. Contamination of bananas with beauvericin and fusaric acid produced by *Fusarium oxysporum* f. sp. *cubense*. Plos One, Vol. 8. 10.1371/journal.pone.0070226.
34. Singh, V.K. and R.S. Upadhyay, 2014. Fusaric acid induced cell death and changes in oxidative metabolism of *Solanum lycopersicum* L. Bot. Stud., Vol. 55. 10.1186%2Fs40529-014-0066-2.
35. Jayasankar, S., R.E. Litz, D.J. Gray and P.A. Moon, 1999. Responses of embryogenic mango cultures and seedling bioassays to a partially purified phytotoxin produced by a mango leaf isolate of *Colletotrichum* gloeosporioides Penz. *In vitro* Cell. Devel. Biol. Plants, 35: 475-479.
36. Mohanraj, D., P. Padmanaban and M. Karunakaran, 2003. Effect of phytotoxin of *Colletotrichum falcatum* went. (*Physalophora tucumanensis*) on sugarcane in tissue culture. Acta Phytopathol. Entomol. Hung., 38: 21-28.

37. Matsumoto, K., M.L. Barbosa and L.A.C. Souza, l995. Race 1 *Fusarium* wilt tolerance on banana plants selected by fusaric acid. Euphytica, 84: 67-71.
38. Borras, O., R. Santos, A.P. Matos, R.S. Cabral and M. Azola, 2001. A first attempt to use a *Fusarium subglutinans* culture filtrate for the selection of pineapple cultivars resistant to Fusariose disease. Plant Breed., 120: 435-438.
39. Chawla, H.S. and G. Wenzel, 1987. *In-vitro* selection for fusaric acid resistant barley plants. Plant Breed., 99: 159-163.
40. Remotti, P.C., H.J.M. Loffler and L. van Vloten-Doting, 1997. Selection of cell-lines and regeneration of plants resistant to fusaric acid from *Gladiolus x Grandifolorus* cv. Peter Pears. Euphytica, 96: 237-245.
41. El Hadrami, A., A. El Idrissi-Tourane, M. El Hassani, F. Daayf and I. El Hadrami, 2005. Toxin-based *in-vitro* selection and its potential application to date palm for resistance to the bayoud fusarium wilt. A review. Comptes Rendus Biol., 328: 732-744.
42. Nedelnik, J. and J. Repkova, 1998. Plant selection *in vitro* for resistance to some pathogens using secondary toxic metabolites. Czech J. Genet. Plant Breed., 34: 69-76.
43. Shahin, E.A. and R. Spivey, 1986. A single dominant gene for *Fusarium* wilts resistance in protoplast-derived tomato plants. Theoret. Applied Genet., 73: 164-169.
44. Kintzios, S., A. Koliooulos, E. Karyoti, J. Drossopoulos, C.D. Holevas, A. Griggoriu and C.G. Panagopoulos, 1996. *In vitro* reaction of sunflower (*Helianthus annuus* L.) to the toxin(s) produced by *Alternaria alternata*, the casual agent of brown leaf spot. J. Phytopathol., 144: 465-470.
45. Ishida, Y. and T. Kumarshiro, 1988. Expression of tolerance to the host-specific toxin of *Alternaria alternate* (AT toxin) in cultured cells and isolated protoplasts of tobacco. Plant Dis., 72: 892-895.
46. Kodama, M., T. Yoshida, H. Otani, K. Kohmoto and L.S. Nishimura, 1991. Effect of al-toxin produced by *Alternaria alternata* tomato pathotype on viability of cultured tomato cells determined by MTT-colorimetric assay. Ann. Phytopath. Soc. Jpn., 57: 663-670.
47. Saito, A., N. Nakazawa and M. Suzuk, 2001. Selection of mutants resistant to *Alternaria* blotch from *in vitro* cultured apple shoots irradiated with X- and γ-rays. J. Plant Physiol., 158: 391-400.
48. Rines, H. and H.H. Luke, 1985. Selection and regeneration of toxin-insensitive plants from tissue cultures of oats (*Avena sativa*) susceptible to *Helminthosporium victoriae*. Theor. Applied Genet., 71: 16-21.
49. Wenzel, G. and B. Foroughi-Wehr, 1990. Progeny tests of barley, wheat and potato regenerated from cell cultures after *in vitro* selection for disease resistance. Theor. Applied Genet., 80: 359-365.
50. Keller, B., H. Winzeler, M. Winzeler and P.M. Fried, 1994. Differential sensitivity of wheat embryos against extracts containing toxins of *Septoria nodorum*: First steps towards *in vitro* selection. J. Phytopathol., 141: 233-240.
51. Hunold, R., H. Hartleb and O.S. Afanasenko, 1992. Resistance against *Drechslera teres* (Sacc.) Shoem. in progenies of *in vitro* selected callus derived plants of barley (*Hordeum vulgare* L.). J. Phytopathol., 135: 89-98.
52. Sayed, A.O., Z.A. Kasem, T.A.R. Abdel-Rahem and A.M. Abdel-Rahem, 2016. *In vitro* technique for selecting onion for white rot disease resistance. Afr. Crop Sci. J., 24: 305-315.
53. Kumar, P., R. Chand, V. Singh and C. Pal, 2017. *In vitro* screening of calli of mungbean to cercosporin, a photoactivated toxin. Int. J. Exp. Bot., 55: 113-121.
54. Lepoivre, P., J. Viseur, K. Duhem and N. Carels, 1986. Double Layer Culture Technique as a Tool for Selection of Calluses Resistant to Toxic Material from Plant Pathogenic Fungi. In: Somaclonal Variations and Crop Improvement, Semal, J. (Ed.)., Springer, Nijhoff, Dordrecht, pp: 45-52.
55. Ahmed, K.Z., A. Mesterhazy and F. Sagi, 1991. *In vitro* techniques for selecting wheat (*Triticum aestivum* L.) for Fusarium-resistance. I. Double-layer culture technique. Euphytica, 57: 251-257.
56. Kleijer, J., N.N. Diop and A. Fossati, 1996. Plant Regeneration and *in vitro* Selection of Triticale. In: Triticale: Today and Tomorrow. Developments in Plant Breeding, Guedes-Pinto, H., N. Darvey and V.P. Carnide (Eds.)., Springer, Dordrecht, pp: 373-377.
57. Esmaiel, N.M., A.A. Al-Doss and M.N. Barakat, 2012. *In vitro* selection for resistance to *Fusarium oxysporum* f. sp. dianthi and detection of genetic polymorphism via RAPD analysis in carnation. J. Med. Plants Res., 6: 3997-4004.
58. Nasir, I.A. and S. Riazuddin, 2008. New approaches to generate disease-resistant gladiolus. World J. Microbiol. Biotech., 24: 367-378.
59. Chopra, V.L., S.B. Narasimhulu, P.B. Kirti, S. Prakash and G. Anuradha, 1989. Studies of Somaclonal Variation in *Brassica* spp. and its Relevance to Improvement of Stress Tolerance and Yield. In: Review of Advances in Plant Biotechnology, 1985-1988, 2nd International Symposium on Genetics Manipulation in Crops, Mujeeb-Kazi, A. and L.A. Sitch (Eds.)., CIMMYT., Mexico and Manila, Philippines, pp: 229-238.
60. Tripathi, M.K., S. Tiwari and U.K. Khare, 2008. *In vitro* selection for resistance against purple blotch disease of onion (*Allium cepa* L.) caused by *Alternaria porri*. Biotechnology, 7: 80-86.
61. Arcioni, S., M. Pezzotti and F. Damiani, 1987. *In vitro* selection of alfalfa plants resistant to *Fusarium oxysporum* f. sp. medicaginis. Theor. Applied Genet., 74: 700-705.
62. Srinath, R. and M. Padmaja, 2000. Selection of chickpea cell lines resistant to culture filtrate of *Fusarium oxysporum* f. sp. *ciceri*. Phytomorphology, 50: 41-46.
63. Jayasankar, S. and D.J. Gray, 2003. *In vitro* selection for disease resistance in plants- an alternative to genetic engineering. AgBiotechNet, 5: 1-5.

64. Rai, M.K., R.K. Kalia, R. Singh, M.P. Gangola and A.K. Dhawan, 2011. Developing stress tolerant plants through *in vitro* selection: An overview of the recent progress. Environ. Exp. Bot., 71: 89-98.
65. Kumar, J.V., B.D. Ranjitha Kumari, G. Sajatha and Enrique Castano, 2008. Production of plants resistant to *Alternaria carthami* via organogenesis and somatic embryogenesis of safflower cv. NARI-6 treated with fungal culture filtrates. Plant Cell Tissue Organ Culture, 93: 85-96.
66. Singh, V.K. and R.S. Upadhyay, 2016. Induction of defence responses by fusaric acid (*Fusarium toxin*) in tomato plant. Proceedings of the 6th International Conference in Agriculture, Environment and Biological Science, December 21-22, 2016, Kuala Lampur, Malaysia, pp: 99-104.
67. Hrubcova, M., M. Cvikrova, L. Meravy, J. Eder and P. Binarova, 1992. Phenolic accumulation and peroxidase activity in *in vitro* selected alfalfa callus cultures resistant to filtrate of *Fusarium* spp. Biol. Plantarum, Vol. 34. 10.1007%2FBF02925869.
68. Ganesan, M. and N. Jayabalan, 2006. Isolation of disease-tolerant cotton (*Gossypium hirsutum* L. cv. SVPR 2) plants by screening somatic embryos with fungal culture ltrate. Plant Cell Tissue Organ Culture, 87: 273-284.
69. Gengenbach, B.G., C.E. Green and C.M. Donovan, 1977. Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Nat. Acad. Sci., 74: 5113-5117.
70. Sacristan, M.D., 1985. Selection for disease resistance in *Brassica* cultures. Hereditas, 103: 57-63.
71. Behnke, M., 1979. Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. Theor. Applied Genet., 55: 69-71.
72. Thanutong, P., I. Furussawa and M. Yamamoto, 1983. Resistant tobacco plants from protoplast-derived calluses selected for their resistance to *Pseudomonas* and *Alternaria* toxins. Theor. Applied Genet., 66: 209-215.
73. Gentile, A., E. Tribulato, Z.N. Deng, E. Galun, R. Fluhr and A. Vardi, 1993. Nucellar callus of 'Femminello' lemon, selected for tolerance to *Phoma tracheiphila* toxin, shows enhanced release of chitinase and glucanase into the culture medium. Theor. Applied Genet., 86: 527-532.
74. Nyange, N.E., B. Williamson, G.D. Lyon, R.J. McNicol and T. Connolly, 1997. Responses of cells and protoplasts of *Coffea Arabica* genotypes to partially purified culture filtrates produced by *Colletotrichum kahawae*. Plant Cell Rep., 16: 763-769.
75. Yang, Z., X. Yang and D. Huang, 1998. Studies on somaclonal variants for resistance to scab in bread wheat (*Triticum aestivum* L.) through *in vitro* selection for tolerance to deoxynivalenol. Euphytica, 101: 213-219.
76. Thakur, M., D. Sharma and S. Sharma, 2002. *In vitro* selection and regeneration of carnation (*Dianthus caryophyllus* L.) plants resistant to culture filtrate of *Fusarium oxysporum* f. sp. dianthi. Plant Cell Rep., 20: 825-828.
77. Gayatri, M.C. and R. Kavyashree, 2005. Selection of turmeric callus tolerant to culture filtrate of *Pythium graminicolum* and regeneration of plants. Plant Cell Tissue Organ Culture, 83: 33-40.
78. Valencia, L.D., C.B. Pascual and E.F. Delfin, 2014. *In-vitro* selection of pineapple cv. 'Queen' with resistance to culture filtrate of *Phytophthora cinnamomi* Rands. Philippine J. Crop Sci., 39: 58-66.
79. Nurcahyani, E., E. Suharyanto, B. Hadisutrisno and I. Sumardi, 2015. *In vitro* selection on fusaric acid of *Vanilla planifolia* plantlets for obtaining a cultivar, which resistant to *Fusarium oxysporum* f. sp. *vanilla*. KnE Life Sci., 2: 617-620.
80. McCoy, T.J., R.L. Phillips and H.W. Rines, 1982. Cytogenetic analysis of plants regenerated from oat (*Avena sativa*) tissue cultures; high frequency of partial chromosome loss. Canad. J. Genet. Cytol., 24: 37-50.