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AFLATOXIN CONTAMINATION IN GROUNDNUT INDUCED BY *ASPERGILLUS FLAVUS* TYPE FUNGI: A CRITICAL REVIEW

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Groundnut (*Arachis hypogaea* L.) is an annual legume which is also known as peanut, earthnut, monkeynut and goobers. It is the 13th most important food crop and 4th most important oilseed crop of the world. Groundnut seeds are a nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as culinary oil. It is also used as animal feed (oil pressings, seeds, green material and straw) and industrial raw material (oil cakes and fertilizer). These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries.
The crop is affected by several diseases like leaf spots, collar rot, rust, bud necrosis, stem necrosis etc. Apart from these, aflatoxin is one of the major problems, produced in the infected peanut seeds by *Aspergillus flavus* Link ex fries and *Aspergillus parasiticus* Speare, particularly at the end of season under drought conditions (Diener *et al.,* 1987). Aflatoxins are highly carcinogenic, immunosuppressive agents, highly toxic and fatal to humans and animals particularly affecting liver and digestive track. Aflatoxin is a potent human carcinogen. It is a naturally occurring toxic metabolite produced by certain fungi (*Aspergillus flavus*), a mold found on food products such as corn and peanuts, peanut butter. It acts as a potent liver carcinogen in rodents (and, presumably, humans). They are probably the best known and most intensively researched mycotoxins in the world. Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. In the present chapter, a detailed account on groundnut aflatoxins induced by *A. flavus* group of fungi was presented.

ECONOMIC IMPORTANCE OF AFLATOXINS

A variety of contaminants are found naturally occurring in foods. Of these, mycotoxins are the major contaminants and 25 percent of foods are contaminated with mycotoxins. Among them aflatoxins are the major mycotoxins produced by toxigenic strains of *A. flavus* and *A. parasiticus* in the suitable environment. Aflatoxins are the secondary metabolites produced by these fungi. Aflatoxins cause economic and trade problems at almost every stage of marketing of groundnut especially during export. Earlier reports indicated that over a decade, the export of groundnut productions from India has declined from 550 metric tons (valued at US$ 42.5 million) to 265 metric tons (valued at US$32.5 million) due to the presence of aflatoxins. Importing countries have prescribed the standards for groundnut. In India permissible level for aflatoxin in groundnut is 30ppb per kg. According to Indian council of medical research (ICMR), Lucknow, 21 % of groundnut and maize samples in India are unfit for human consumption due to aflatoxin contamination. In order to protect the international trade, in 29th meeting, codex committee on food additives and contaminants (CCFAC) a draft level of 15microgram per kg for total aflatoxin in peanuts intended for further processing was proposed as the maximum level. If this level is applied, 37 percent of our groundnut samples are rejected. In Andhra Pradesh groundnut samples contain 15-19 percent excess aflatoxin than the permissible level.

Various surveys conducted in different parts of India have shown a range of aflatoxin levels in peanut food products including raw peanut kernels (0.8 to 2200 µg/kg), edible flour (0 to 200 µg/kg), unrefined oil (up to 786 µg/kg) and peanut cake (27 to 1122 µg/kg) depending upon the agro climatic location and storage conditions (Ghewande, 1997).
Due to the contamination of aflatoxins, the peanut is considered as a high risk commodity. The problem of aflatoxin contamination is worldwide but in India, the poor harvesting practices, high temperature, high moisture levels and post harvest practices are conducive for fungal growth, proliferation and aflatoxin contamination. If other conditions are favourable, aflatoxin can be produced at temperature ranging from 11 to 40°C although 25 to 30°C is the optimal range (Diener and Davis, 1987). Aflatoxins are produced under drought conditions in pre-harvest peanut crop. To manage the aflatoxin problem in groundnut, identification of resistant crop varieties is the best approach, but none of the varieties screened so far are completely immune to aflatoxin production.

DESCRIPTION OF PATHOGEN

A. flavus is a saprophyte or facultative parasite. It produces green colored conidia on artificial medium. The sterigmata are typically biseriate, conidia conspicuously echinulate, conidiophores heavy walled, coarsely roughened and sclerotia may be produced.
Holbrook *et al* (2000) prepared *A. flavus* inoculum by organic matrix method. Ten days old green conidia of *A. flavus* was suspended in sterile distilled water, 10 ml of this inoculum suspension was added to 114 g of broken Bajra seeds and incubated or for 3 days at 25-30˚C.

SCREENING STUDIES

Mixon and Rogers (1973) developed a new *in vitro* seed colonization procedure for screening the groundnut genotypes against *A. flavus.* Their results indicated that Valencia type genotypes viz., PI337394F and PI337409 were resistant to two toxin producing strains of the fungus.
Priyadarshini and Tulpule (1978) studied the reaction of different varieties of maize and groundnuts and stated that there is no direct correlation between fungal growth and aflatoxin production, suggesting that the genotypes produced different amounts of aflatoxin per unit growth of the fungus.
Levels of *A. flavus* infection and aflatoxin contamination are related primarily to environmental conditions especially to drought stress during pod maturation. Hence, the levels of *A. flavus* seed infection cannot be directly correlated to the aflatoxin production (Davidson *et al.,* 1982).
Kisyombe *et al* (1985) evaluated 14 peanut genotypes in rain shaded field microplots against to *A. parasiticus* and found that genotypes J-11 and Lampang were resistant to this fungus under both dry and moist field conditions. They also evaluated 34 genotypes for dry seed resistance in laboratory and found that there was no correlation between genotype for resistance to dry seed infection and resistance under field conditions.
Blankenship *et al* (1985) evaluated groundnut genotypes to *A. flavus* infection under laboratory conditions and found that all were resistant. However, these genotypes when evaluated under field conditions by imposing the drought and temperature conditions were found to be susceptible. Resistance to aflatoxin contamination in peanut operates at three levels, resistance to fungal invasion at the pod wall at the seed coat and resistance to aflatoxin production in the cotyledinary tissue. (Mixon, 1986). Resistance to aflatoxin contamination in peanut operates at three levels, resistance to fungal invasion at the pod wall, at the seed, coat and resistance to aflatoxin production in the cotyledinary tissue (Mixon, 1986).
Kiran-kalia *et al* (1988) evaluated 53 groundnut cultivars and found that high yielding lines were susceptible to invasion by *A. flavus* and aflatoxin contamination. These results also indicated that line OG 35-1 showed highest resistance with low yield potential and J-11 showed resistance to aflatoxin production and moderately susceptible to *A. flavus* invasion.

Waliyar and Bockeiee-morvan (1989) reported significant varietal differences in levels of seed invasion by *A. flavus* at harvest. They also showed that under field conditions, resistance was positively correlated with *in vitro* seed colonization.The commercially grown CVS 55-437, 73-30 and 73-33 exhibited moderate to high levels of resistance to infection. The cultivar resistant to seed invasion had lower frequency of *A. flavus* counts in their rhizosphere compared to those of susceptible cultivars. Naguib *et al* (1990) reported that all 21 cultivars of groundnut genotypes from ICRISAT and four from Egypt supported the production of aflatoxin B_1 and B_2 in seed when inoculated with *A. flavus*

Desai *et al* (1991) tested 39 different groundnut varieties and breeding lines to *A. flavus* infection and found that tested groundnuts were significantly differed in infection and aflatoxin production, infection and seed colonization were strongly correlated and no correlation was found between infection and aflatoxin content.

Ghewande *et al* (1993) screened 38 groundnut genotypes, under artificially inoculated conditions and reported that there was a significant correlation between infection, colonization and aflatoxin content. However, there was no correlation between sugar content and infection, colonization and aflatoxin content.

Waliyar *et al* (1994) evaluated 25 groundnut lines and reported that 55-437, J-11 and PI337394 were least infected, ICGV-87084, ICGV-87094, ICGV-87110 were resistant and var-29 showed a high percentage of infection with low aflatoxin contamination.

Anderson *et al* (1995) evaluated 12 potentially resistant genotypes for pre-harvest aflatoxin contamination and found that none of the genotypes were more resistant ($P<0.05$) to pre-harvest aflatoxin contamination than the genotype florunner.

Anderson *et al* (1996) developed an effective procedure i.e., *in vitro* seed colonization for screening the individual plants for resistance to invasion by *A. flavus.*

Chiou *et al* (1999) studied mould infection and aflatoxin contamination of crops by *A. flavus* and *A. niger* inoculation either with *A. niger* alone or combined with *A.flavus* resulted in various levels of seed and seedling mortality and lower yields of peanut pods than yields of other inoculation treatments. He also showed that colonization and aflatoxin content were independent of artificial inoculation.

Holbrook *et al* (2000) evaluated 20 genotypes of groundnut having drought tolerance and susceptibility. These results indicated that susceptible genotypes had greater preharvest aflatoxin contamination and drought tolerant genotypes had less preharvest aflatoxin contamination. Mohan *et al* (2003) screened 13 confectionary groundnut genotypes against *A. flavus* seed colonization. None of the genotypes of cultivated groundnut showed stable resistance to *A. flavus* although there is certain degree of resistance to seed colonization in the genotypes studied.

ESTIMATION OF *ASPERGILLUS FLAVUS* POPULATION IN SOIL

Pitt (1980) estimated *A. flavus* population from 300 soil samples of Australia by using AFPA medium. Nearly 90 percent of the samples from soil in which groundnuts had grown showed in 100 to 5000 spores per g. of soil. Among 30 virgin soil samples, only 3 samples contained *A. flavus* one sample contained 100 spores per gm of soil. Thus it is concluded that groundnut cultivation increases the fungal population in soil.

Okazaki *et al* (1992) estimated the population of *A. flavus* @ 30±9.7 and 11±2.3 propagules /gm of soil in Kumamoto and miyakonojo areas respectively and also found that the fungal populations widely distributed in fields.

Lee and Chuang (1993) estimated aflatoxin producing strains of *A. flavus* in soil by using the differential medium i.e., aflatoxin producing agar (APA). The highest frequency of *A. flavus* population was found at a depth of 5-10 cm followed by 10-15 cm, but population was low at upper layers (0-5 cm) and least in deeper layers (15 -20 cm). Aflatoxin producing strains were in the range of 45-72 percent of the total *A. flavus* population of the soil and more strains were isolated from soil than pod shells and kernels.

AFLATOXIN ESTIMATION BY ELISA

Fan and Chu (1984) used indirect ELISA technique for the estimation of aflatoxin B_1 in corn and peanut butter. Ram *et al* (1986) detected aflatoxin B_1 in maize and cotton seed by ELISA and found that the toxin is in the range of 7 to 422 µg/kg and 7 to 3258 µg/kg respectively. Cole *et al* (1988) estimated the aflatoxin content in 152 groundnut grade samples by ELISA and high performance liquid chromatography (HPLC) and found that 14 percent of the samples contained 26-2543 µg kg^{-1} aflatoxin.
Anjaiah *et al* (1989) used competitive direct ELISA for estimation of aflatoxin B_1 in naturally contaminated groundnut seed samples and concluded that this assay is more rapid and less expensive than physicochemical methods. Moreover, it can be used to detect as low as 50 pg of aflatoxin B_1. Candlish *et al* (1987) estimated the aflatoxin content in groundnut kernels, groundnut butter and maize by enzyme immunosorbent assay (EIA) and Thin layer chromatography (TLC) and observed the positive correlation between EIA and TLC.
Cole *et al* (1988) estimated the aflatoxin content in 152 groundnut grade samples by ELISA and HPLC (High performance liquid chromatography) and found that 41 percent of the samples contained 26-2542 µg/kg aflatoxin. The results of ELISA and HPLC agreed in 98.6 percent of the composite lot analysis with the detection of 20 µg/kg or greater.
Mortimer *et al* (1988) analyzed the aflatoxin content in groundnut butters (129 samples) by ELISA and these results showed that 6.2 percent of samples contained aflatoxin over 10 µg per kg, 8 percent contained 2.5 to 10 µg per kg and in the remainder (86 percent) does not contain aflatoxin. These results concluded that ELISA is a faster than conventional approaches.
Chu *et al* (1988) analyzed aflatoxin content in groundnuts and groundnut products by Radio immuno assay (RIA), which had sensitivity in the range of 0.1-0.5 ng, whereas ELISA, had sensitivity in the range of 2.5-25pg per assay. Simple and quick immunoassay (ELISA) protocols for monitoring aflatoxin B_l in groundnuts was developed that require less than 1h to complete and detect 5 to 10µg/ kg product.
Park *et al* (1989) estimated aflatoxin B_l in 12 raw and roasted ground nuts and maize containing natural aflatoxins and also supplemented with aflatoxin B_l. Overall correlation was good between ELISA and TLC results for maize and roasted groundnut products. It is concluded that the ELISA method is approved interim official first action due to its simplicity and fast determine the presence or absence of aflatoxin B_1 at a concentration of 20 ng/g in maize and roasted groundnuts.
Park *et al* (1989) recommended the use of ELlSA, as a screening method to determine the presence or absence of aflatoxin B_l at a concentration of ≥15 mg per g in cotton seed products and mixed feed. Figuiera *et al* (1990) estimated the aflatoxin B_l in groundnuts, Brazil nuts, Almonds, Hazelnuts and Walnuts by ELISA and recommended an alternate method to the already adopted TLC method.
Azer and Cooper (1991) used ELISA system and HPLC method simultaneously to analyse 178 samples of foodstuffs for total aflatoxins. High correlation coefficient values obtained between results of two methods with nuts, nut products, groundnuts and poor correlation for cereals and grain samples.
Mehan *et al* (1992) reported the significant differences in the production of AFB_1 aflatoxin between the accessions of wild *Arachis* spp. and 5 of groundnut samples. Patey *et al* (1992) used ELISA technique for the quantification of aflatoxin content in peanut butter. Ramakrishna and Mehan (1993) utilized indirect and direct ELISA for the determination of aflatoxin B_1 in groundnut and detected as low as 20 pg per well. It is also concluded that both direct and indirect methods are useful for routine analysis of aflatoxin B_l in groundnuts.
Aldao *et al* (1995) quantified the aflatoxin B_1 by an indirect ELISA in groundnut samples and observed the cross reactivity of antibodies with aflatoxin B_2, G_1 and G_2. Sylos *et al* (1996) estimated aflatoxin content in 10 samples of groundnut and 9 samples of maize by ELISA and mini column chromatography and detected >20 µg/ kg toxin in 50 percent groundnut seeds and none in maize samples and also ELISA has taken less time to complete than mini column chromatography. Zhang *et al* (1997) collected 246 samples of corn from different areas and analyzed for aflatoxins and fuminosins by ELISA and more toxins were detected in areas of high oesophageal cancer (HEC) and low toxins were detected in HEC low risk areas. Reddy *et al* (2001) estimated AFB_1 in different grades of chilli samples by indirect ELISA and showed that 59 percent of samples were contaminated with $AFB_1$and 18 percent contained the toxin at non permissible levels. Maximum percentage of chilli pods showing AFB_1 levels higher than 30 µg per kg in grade 3.

BIOCHEMICAL CHARACTERS

Total phenols, Total proteins, Sugars

Bhatia *et al* (1972) concluded that ability of tomato plants to resist infection by *Alternaria solani* depend on the quality of phenolics in the leaf, stem and roots of the plants. Higher amounts of total phenolics were found in the resistant variety than in susceptible variety.

Deshpande and Pancholy (1979) evaluated five genotypes against *A. flavus* and found that two of them are highly resistant to colonization than others and noted a reduction in oil and protein content and rapid increase in free fatty acids and changes in aminoacid composition. Chattopadhyay and Bera (1980) found an increase in phenolics and phenol oxidase activity in resistant variety of rice leaves following infection with bacteria and fungi. Polyphenol oxidase converts phenols to Quinones, which may be responsible for general resistance in higher plants towards bacteria and fungi.

Mishra *et al* (1980) reported that the total phenols and O-dihydric phenols were higher in *C. graminicola* resistant sorghum varieties than susceptible ones. Bilgrami *et al* (1983) conducted an experiment on chemical changes in dry fruits due to aflatoxin elaboration by *A. flavus* and found that significant loss in the quantity of total, reducing and non-reducing sugars as well as ascorbic acid level. An increase in total protein and phenol content was also observed in *A. flavus* infected coconut almond, cashew nut, walnut and mekhana.

Anahosur *et al (*1985) found that higher levels of sugars and phenolics were present in sorghum genotypes resistant sorghum to *Macrophomina phaseolina* than in susceptible ones. The results of Shree and Reddy (1986) indicated the large amount of total phenols, reducing sugars, free aminoacids and proteins in resistant (CSH6 and 148) lines of sorghum against *Helminthosporium turcicum.* Basha and Pancholy (1986) inoculated the peanut seeds with 4 different *Aspergillus* lines and infected seeds showed decrease in oil, iodine value, soluble carbohydrates and protein content. Two dimensional gel electrophoresis showed gradual disappearence of a high molecular weight polypeptide of 70KD. Several polypeptides with molecular weights between 16KD and 34KD were also appeared 9 days after inoculation.

Gupta *et al* (1987) analyzed the leaf extract from sesame varieties susceptible (Till No.1 and HT-l) and resistant (RT-4-6, HT-24) to *Alternaria sesami* for total phenols. These results indicated that in all varieties, the total phenols were decreased. Phenols were higher in resistant cultivars than susceptible one-depending on age of plants and variety.

Yadav and Mishra (1987) observed that there was increase in total phenols, soluble proteins and peroxidase enzyme activity in rice tungro virus infected TN 1 (susceptible) and IR-8 (tolerant) rice varieties, while in saket-4 (resistant) variety, it remained unaltered.

Raguchander *et al* (1988) studied the resistance mechanism of triticale cultivars against leaf blight fungus *Bipolaris sorokiniana.* They observed lower levels of phenols, sugars and proteins in susceptible cultivars than in resistant cultivars. Mandavia and Parameswaran (1993) reported higher levels of amino acids and phenolics in the resistant cultivar (PLJ-1) of Limabean infected with stem rot.

Bhatia and Takur (1994) noted an increase in protein content of pearl millet in leaves and stem but decrease in roots at all stages of growth in susceptible cultivar in relation to downy mildew pathogen.

Chowdhury (1995) recorded higher levels of phenolics, proteins and peroxidase and polyphenol oxidase activities in IAA treated groundnut plants against *Puccinia arachidis* compared to untreated plants.

Rajivkumar and Singh (1996) observed that the polyphenol content in healthy sunflower leaves was higher and increased at 40 and 70 days after inoculation with *Alternaria.* The results of Bhite *et al* (1997) revealed that resistant cultivars contained significantly higher amount of total phenolics and chlorogenic acid in leaves compared to susceptible cultivars of pigeon pea infected with sterility mosaic virus.

Bhavani *et al* (1998) reported high protein content in mosaic virus infected sunflower leaves compared to healthy leaves. Rathi *et al* (1998) observed higher amounts of total phenols in powdery mildew resistant pea leaves than susceptible varieties. After infection, the phenolic contents were more in resistant varieties than in susceptible ones.

Grewal *et al* (1999) reported the higher concentration of total sugars, total phenols and proteins in resistant varieties of wheat compared with the susceptible varieties against *Neovossia indica* causing kernal bunt.

Maintenance of high protein and phenolic contents in resistant genotypes of groundnuts when infected with *Cercospora* was reported by Bera *et al* (1999).

Kernel moisture and pod wall moisture

Cole *et al* (1985) suggested that after seed invasion by *A. flavus,* growth of the fungus and aflatoxin production did not occur until the natural resistance mechanisms in the kernel had broken down as a result of environmental (water and temperature) stresses.

Wotton and Strange (1987) suggested that phytoalexin production, under high moisture conditions may provide a resistance mechanism to prevent spore germination and hyphae extension of *A. flavus.* Cole *et al* (1993) found that enhanced resistance of peanut genotypes was partially associated with improved drought tolerance as measured by the ability to maintain high kernel moisture under extended drought conditions. Holbrook *et al* (1994) evaluated pre-harvest aflatoxin resistance in a set of groundnut genotypes that had reputed variations in drought conditions.

Nageswara Rao *et al* (2001) have suggested that management of drought, by either escape tolerance or avoidance mechanisms may therefore have a significant impact on a genotype ability to reduce aflatoxin contamination.

ANALYSIS OF TOTAL GROUNDNUT SEED PROTEINS BY SDS-PAGE

Cherry *et al* (1975) analyzed the proteins of groundnut seeds seven days after infection with *A. pasasiticus* and compared with uninoculated seeds by gel electrophoresis. They reported that soluble extracts from inoculated peanuts showed that proteins were hydrolyzed to many small molecular weight components, which eventually disappeared as fungal growth progressed. A corresponding increase in quantity of most of the amino acids was observed shortly after the inoculation of the peanut. It was concluded that infection of fungus initiated a sequence of events where by proteins were hydrolyzed first to small polypeptides and (or) insoluble components, then to free aminoacids.

Cherry *et al* (1976) examined proteins and total aminoacids in peanuts inoculated with *Aspergillus oryzae* at various time intervals over an 18 day test period. Aquarius buffer soluble proteins were declined first and increased rapidly during test period. Gel electrophoresis showed that proteins were converted to aminoacids. These aminoacid profiles distinguished from inoculated and uninoculated seeds because of increased levels of aminoacids in inoculated seeds than un inoculated seeds.

Bianchi-Hall and Thomson (1979) analyzed fifty-eight accessions of wild peanuts for seed storage proteins by SDS-PAGE. Many dark and lightly stained bands were observed, only the major bands corresponding to the acidic and basic proteins were compared. One to five bands were observed with regard to acidic arachin and 2 to 5 bands were observed with regard to basic arachin proteins. It helps in clear assessment of the large amount of variability in protein composition in peanuts and should aid in defining phylogenetic relationships in *Arachis*.

Krishna *et al* (1986) observed the protein profiles of six diploid species of *Arachis monticola* and five accessions of *Arachis hypogea* and found highly conserved nature of "arachis" polypeptide in all the accessions. Lanham *et al* (1994) screened 72 accessions representing 22 spp of *Arachis* for seed storage protein variability. They detected variation among section, genome types and spp. They could differentiate the two sub spp. of *A. hypogaea* (fastigate and hypogaea) based on presence or absence of 44 KD or 42 KD polypeptides. The 44 KD band was found in spp. fastigated, while 42 KD band in spp. hypogea only.

Lavon *et al* (1999) determined the SDS-PAGE pattern of soluble and chloroplast membrane proteins in rough lemon *(Citrus volkameriana)* under K, Mg and Ca deficiencies. The SDS-PAGE patterns of soluble and chloroplast membrane proteins did not reveal major quantitative changes and they concluded that the data do not demonstrate a general close link between chlorosis, minerally deficient citrus leaves and nitrogen metabolism.

Naik and Kole (2001) analyzed the total seed proteins of 37 mungbean genotypes by SDS-PAGE. These results showed the protein bands of varying intensity and heterogeneous molecular weights over five zones i.e., A to C within a molecular range of 17.4 to 75.0 KD.

ISOZYMES

Brim *et al* (1969) observed the large differences in peroxidase zymograms among different soybean tissues and also reported three quantitative genotypic differences involving peroxidase isozymes. Seevers *et al* (1971) separated peroxidase isozymes from healthy and inoculated lines of wheat near isogenic lines for resistance and susceptibility to race 56 of *Puccinia graminis tritici* by gel electrophoresis. Among the 14 isozymes detected in both healthy and infected leaves, increase in only one (isozyme 9) were associated with the development of resistant disease reaction at 20°C.

Cherry *et al* (1978) analyzed the different isozymes like esterases, leucine aminopeptidase, gluconase, alcohol dehydrogenase and alkaline and acid phosphatase pattern in extracts of groundnut seeds infected with aflatoxicogenic strains. These results indicated that the zymograms did not differ significantly from patterns of seeds infected with non aflatoxicogenic strains.

HammerSchmidt *et al* (1982) observed the enhancement of peroxidase activity in second leaf of cucumber, when first leaf was inoculated with *Colletotrichum legenarum.* This increased activity was supposed to be partially associated with the fastest moving acidic isozymes observed when separated by PAGE. Lima (1982) observed considerable polymorphism among esterases in citrus by electrophoretic studies with leaves of young shoots of 161 taxa. Arora and Bajaj (1985) recorded the variability in peroxidase enzyme banding pattern in hypocotyls of mungbean after infection with *Rhizoctonia solani.*

Puchalski *et al* (1986) observed the induction of three peroxidase isozyme in rye seedlings after inoculation with *Fusarium nivale* and suggested that the isozyme band P x 4 could be used as a maker in identifying resistant genotypes.

Chahal *et al* (1988) investigated the peroxidase isozyme pattern among downy mildew resistant and susceptible pearl millet lines and healthy plants, which indicated the involvement of C_5, C_6 and C_9 isoperoxidases in developing resistance. Uta Gireshammer and Wynne (1990) surveyed 61 US cultivars, one breeding and 6 exotic lines of groundnut for 25 enzyme systems using horizontal gel electrophoresis. Polymorphism was consistently observed with glutamate oxalo acetate transaminase (GOT), phosphohexoisomarase (PHI) and isocitrate dehydrogenase (IDH) indicating limited variability.

Gillikin and Graham (1991) reported that majority of the peroxidase activity in soybean seeds was localized in the seed coat. It is having isoelectric point (PI) and molecular weight of 37 KD similar to the properties of glycoprotein. Lacks and Stalker (1993) evaluated 33 South American groundnut accessions from six countries for 18 isozymes against early leaf spot. Out of these, the polymorphism was observed among glutamate oxaloacetate transaminase (GOT), phospho-hexoisomerase (PHI) and isocitrate dehydrogenase (IDB). A specific PHI band was observed in all three hybrid lines with early leaf spot resistance.

Subash Chandrabose and Ranjan (2000) analyzed the banding pattern of peroxidase isozyme in 45 and 60 days old tomato leaf samples against *Ralstonia Solanacearum* (bacterial wilt) by using PAGE. They reported that the zymograms of PR x -7 (Rm = 0.361) and PR x -8 (Rm =0.381) in 45 days old samples and PR x -5 (Rm =0.297) in 60 days old samples could be used as a markers to identify resistant and moderately resistant varieties.

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