



REVIEW ARTICLE

Genetic Transformation of *Arachis hypogaea* Using Novel Genes Conferring Fungal Resistance-A Review

Shahana Chowdhury¹, Anamika Datta², Manzur-E-Mohsina Ferdous³ & Dwaipayan Sinha^{4*}

¹Department of Biotechnology, German University Bangladesh, Gazipur-1702, Bangladesh

²Department of Stem Cell Biology, Kyushu University, Fukuoka, 819-0385, Japan

³Department of Plant and Environmental Sciences, Copenhagen University, Højbakkegård Allé, 2630 Taastrup, Denmark

⁴Department of Botany, Government General Degree College, Mohanpur, Paschim Medinipur, West Bengal-721 436, India

*Email: dwaipayansinha@hotmail.com



ARTICLE HISTORY

Received: 23 September 2021

Accepted: 16 January 2022

Available online

Version 1.0 (Early Access): 06 March 2022

Version 2.0: 01 April 2022



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonpublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, etc. See https://horizonpublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (<https://creativecommons.org/licenses/by/4.0/>)

CITE THIS ARTICLE

Chowdhury S, Datta A, Ferdous M E M, Sinha D. Genetic Transformation of *Arachis hypogaea* Using Novel Genes Conferring Fungal Resistance-A Review. Plant Science Today 9 (2): 405–420. <https://doi.org/10.14719/pst.1504>

Abstract

Peanut (*Arachis hypogaea* L.) or the common 'peanut' is a worldwide popular, affordable food containing high protein, calories, vitamins and minerals. Several biotic and abiotic stresses are responsible for reaching the expected production of peanuts worldwide. Especially, the fungi are the major constraints that not only hamper the production but also that is deadly health hazardous for both human consumption and poultry-livestock. Approaches from various dimensions like cultural management, diseases free cultivar development, hybridization, tissue culture and genetic transformations have been tried to overcome such challenges. This review epitomizes the total scenario from the plant physiological basis of fungal diseases to the peanut development approaches, which aimed to develop a concrete understanding of sustainable management of peanut production. Comparisons of Genetic Engineering methods such as *Agrobacterium*-mediated and direct gene gun (particle bombardment-mediated) with traditional hybridization have been compiled here, furthermore, candidate genes transformed to achieve fungus-resistance in peanuts have been listed up to provide an overview. Along with, the limitations of transformation attempts and the techniques for improvisation of transformation techniques have been discussed in sustainable peanut production. This study provides comprehensive information on fungal-resistant peanut development so that, further research in this arena could be guided in an integrated way, which may serve for the thrust of sustainable improvisation in peanut cultivation.

Keywords

fungus; genetic engineering; transformation; aflatoxin; *Arachis hypogaea*.

Introduction

Peanut or *Arachis hypogaea* is a tropical leguminous plant having its origin in central Brazil of South America with archaeological records of domestication in Argentina, Bolivia, Peru and Mexico (1). It is presently grown from 40°S to 40°N in warmer locations of tropical, subtropical and Mediterranean climates, where soils are light, neutral to alkaline and rainfall exceeds 400 mm per year (2). The term *Arachis* had its genesis from the Greek word "arachos", which means a 'weed', and *hypogaea*, meaning underground chamber, i.e. in botanical terms, a weed or plant which fruits below the soil surface. Groundnut and peanut are the two most common names of this plant (3). The plant is generally included amongst oilseeds due to its high oil content. Among the oilseeds, worldwide diversifying uses and popularity of peanuts have made it more significant and relevant to the livelihood of a

substantial proportion of the world population. Peanut is an important source of protein, fibres and is widely used for large-scale production of peanut butter, confections, various types of snacks, preparation of soups and desserts (4). Among the numerous varieties of peanuts, 4 market type varieties namely the Runner, Virginia, Spanish and Valencia have worldwide cultivation (5).

Peanut farming faces several contrivances among which the fungal infection forms the major biotic factor hindering the cultivation and overall yield (6). Fungal diseases are known to affect all parts of peanuts namely stem (7), leaf (8) and root (9). These diseases have resulted in yield (10) and economic loss (11) throughout the globe. Several conventional practices such as crop rotation and crop management practices (12) were initially used to control the fungal pathogen. This was accompanied by the use of fungicides for the control of fungal pathogens which often negatively impact the ecosystem (13). In addition to it, indiscriminate and prolonged use of fungicides also resulted in the development of fungicide resistance in the pathogens and ultimately loss of efficacy of the fungicides (14). Moreover, conventional crop rotation practices are also not effective for pathogens that have a wide host range with long-surviving structures such as oospores, chlamydospores and sclerotia (15). Such situations urged plant scientists to rely on biotechnological approaches for controlling fungal pathogens through transformation techniques. These transformation techniques enable the plant to boost its defense system through an expression of a single or a set of foreign genes transferred from a resistant variety or altogether from a different plant. Besides, transformation approaches largely boosts the plant defense system by fortifying various recognition machinery and up-regulating pathways required for defense reaction (16). In this review, an attempt has been made to highlight the various transformation approaches studied towards the development of fungal disease resistant peanut. Several possibilities for disease resistance in peanuts have also been explored. Nonetheless, this review has also come across the limitations of present transformation approaches. Overall, this study presents a synopsis on the transformation of peanut for fungal resistance aimed at developing a fungus-resistant transgenic cultivar.

Materials and Methods

An extensive survey has been made using the internet as a platform and PubMed, Medline, Scopus, and Google scholar as the search engines. Relevant research papers and review articles were short listed and important information were pooled in framing the article.

Results

Description of Plant

Arachis is a perennial legume, 30-50 cm tall, having tetrafoliated, paripinnate, stipulated leaves borne spirally in a 2/5 phyllotaxy. Each leaf is 2-8 cm in length and 1 to 4 cm in width. The inflorescence is of modified sessile papilionaceous type, 2-4 cm in width borne on axils of leaves on primary or secondary branches, spike-like, simple, or compound monopodia with up to five flowers in each node. The flowers are stalked due to the presence of a tubular hypanthium or calyx tube. The flower is subtended by a bract with a second bract on the inflorescence branch. The calyx is two-lobed while the corolla is a papilionaceous type consisting of five petals. Stamens, 10, monadelphous, are with a staminal corona surrounding the ovary (17). The important adaptive feature of groundnut is the formation of an elongated structure called 'peg'. It develops after double fertilization as a result of the elongation of cells of intercalary meristems present at the base of the ovary. The peg is positively gravitropic and moves towards the soil, penetrates it, and forms subterranean pods (18).

Challenges of peanut cultivation

Peanut cultivation faces tremendous challenges by abiotic stresses like temperature, drought, cold, etc. Different fungal and viral agents with insects like pod borer etc. exemplify biotic stresses. The fungal diseases cause substantial harm to the groundnut crop. Due to their detrimental symptoms, it affects the yield of the plants. Moreover, some of the fungal pathogens especially *Aspergillus flavus* produce aflatoxin which is highly harmful to humans. This makes the crops unsuitable for consumption which ultimately leads to financial loss. Thus, the loss of fungal attack can be expressed both in terms of yield and money, both being interrelated to one another.

Challenges of peanut cultivation

In India, the loss of groundnut yields due to leaf spots have been in the range of 15-59% (19). The late leaf spot and the rust constitute important fungal-foliar disease and account for a reduction in a pod and haulm yield by 70% and *in vitro* digestibility of haulms by 22% (20). A survey report from the Junagadh district of Gujrat, India, states a yield loss of 435 kg per ha of groundnut due to dry root rot caused by the fungi *Macrophomina phaseolina* and this corresponded to plant mortality of 29.3% (21). Besides, root rot disease resulted in an economic loss of 20-30% in dry warm conditions (22). Meanwhile, Northern Ghana faces pod yield losses ranging from 9.7 to 81.2% due to early and late leaf spot diseases (23). In the United States of America, the pod rot disease was reported to cause a loss of 20% during the 1960s and 1970s (24). However, in the cases of pod rot, the yield loss is difficult to be determined accurately due to the absence of above-ground parts but losses of as high as 80% have been reported (25).

The loss of yield in groundnut due to defoliation can be correlated with early and late leaf spot diseases of the plants. It was observed that the yield loss was 2.2 to 2.8% per 10% increase in defoliation for levels up to approximately 95% after which the yield loss was found to be exponential (26). In addition, the rust disease of groundnut caused by *Puccinia arachidis* has been reported to cause a loss of 50% (27). Though, the losses can vary in a range of 40% to 70% in favorable conditions and the presence of susceptible cultivars (28). The stem rot of groundnut caused by *Sclerotium rolfsii* may result in as much as 80%

<https://plantsciencetoday.online>

of yield loss though in general losses less than 25% are more common (29). It is also reported that there is a yield loss of 10% in the case of groundnut suffering from *Sclerotinia blight* and in severe cases the loss might be as much as 50% (30). *S. rolfisii* is also reported to cause indirect loss such as reduction in dry weight and oil content of groundnut kernels along with reduction of quality of fodder and pod and incurred a loss of 43 million US dollars annually (31). Several species of *Pythium* have been reported to be the causal organism of vascular wilt, damping-off and root rot of peanut (32).

Apart from the yield and financial loss, the aflatoxin contamination in groundnut is one of the serious matters of concern. Aflatoxin is a carcinogenic mycotoxin that is synthesized by *Aspergillus* fungi and is reported to contaminate a substantial proportion of the world's food (33). Several reports stated the presence of aflatoxin in groundnut. A report states the presence of 158 ng g⁻¹ of aflatoxin B1 in the groundnut cake while the concentration of aflatoxin in the seeds was as high as 3135 ng g⁻¹ in samples from eastern Ethiopia (34). Another study reported the presence of 39 µg kg⁻¹ of aflatoxin in the groundnut from Zambia (35). In South Africa aflatoxin of much higher concentration has been reported in groundnut butter served to the children (36). These contaminations have resulted in economic losses as greater inputs are required for sorting and cleaning up processes. It is estimated that in Benin, the higher purchasing price of clean-up peanuts results in an estimated loss of net returns of 62.30\$ per kg (37). This massive amount of yield loss every year has been urged for measures to prevent or control fungal attacks by conventional or scientific approaches.

Cultural management practice to prevent diseases of peanut

Conventional management practices have been adopted by the farmers to control several diseases of peanut. These methods create an atmosphere that is conducive for the better growth of plants and fortify them to fight against fungal pathogens. The most effective fungal pathogens management of peanuts is the cultural practice which is in concert with chemical control methods. Crop rotation with non-host crops, soil solarization, soil team sterilization (15), eradication of secondary collateral host, burning off old livestock and any remaining plants of previous cultivation in the fields (38) to reduce fungal inocula are the most common cultural management practices. In addition to it, utilizing those host resistance is a significant way in disease management. The inbuilt defense system of the plants allows the farmers to concentrate on other aspects of crop management for maximum return through a sustainable agricultural system (39). Table 1 describes the selected resistant varieties of peanut against fungal pathogens.

The dense canopy of the plant produces a favorable environment for disease incidence by maintaining a humid condition. In this regard, many soil-borne pathogens can be inhibited by maintaining optimum and controlled watering conditions. Since some of the pathogens produce motile zoospores overwatering requires should be avoided (32). The crop rotation is also an effective tool for the control of the fungal disease of peanuts. It is reported that rotating

peanuts with corn, sorghum and other pasture grasses reduce infection from *Rhizoctonia solani* (43). Improving soil

Table 1. Selected fungal resistant varieties of peanut

Fungal pathogen	Peanut varieties	References
<i>Sclerotium rolfisii</i>	UF-MDR-98	(40)
	Georgia-03L and CC650	(41)
	DP-1	(42)
<i>Pythium</i> sp.	Toalson	(25, 43)
<i>Sclerotinia</i> sp.	Tamspan 90	(44)
	Virginia type	(45)
<i>Verticillium</i> sp.	Flavor Runner 458	(46)

fertility may directly affect pathogens, improve crop production by limiting its susceptibility, or increase microbial growth (47-49). For example, the application of nitrogen in the soil reduces the severity of *S. rolfisii* (50) whereas potassium inhibits the growth of *R. solani* and *Sclerotinia sclerotiorum* (51). Calcium is also reported to decrease the occurrence of *Pythium* and *Rhizoctonia*-induced pod diseases in the plant (52).

In the Indian subcontinent, agriculture depends on the use of a wide range of synthetic chemicals, including insecticides, fungicides, herbicides and other pesticides (53, 54). Among the synthetic chemicals, fungicides are antimycotic compounds and are generally used as sprays or dust to control pathogenic fungi. In peanuts, fungicides are frequently used for the control of various pathogens. In the United States, chlorothalonil is widely used to control early leaf spot (*Cercospora arachidicola*) late leaf spot (*Cercosporidium personatum*) and rust (*Puccinia arachidis* Speg) for the last 30 years (55). In addition, prothioconazole, fluxapyroxad and pyraclostrobin have been tested effectively against *Nothopassalora personata*, the causal organism of late leaf sport of peanut (26). To control stem rot disease, caused by *S. rolfisii*, azoxystrobin in combination with tebuconazole is used (56). Another study reported the use of pyraclostrobin, penthiopyrad, and prothioconazole to control the infection of *Cercospora arachidicola* in a systemic mode (57). However, continuous use of fungi results in several deleterious effects on the environment. Firstly, there are reports that fungicides biomagnify in various levels of the trophic system (58-61). In humans, tebuconazole is reported to disrupt the function of the human placental trophoblast cell (62). Reports state that mancozeb complicates nonalcoholic fatty liver disease in humans (63) and is also toxic to mammalian granulosa cells (64). The soil microbiota is also harmed by the application of fungicides which results in a change of soil microbe composition (65). All these harmful effects of fungicides accompanied by inconsistent results of traditional methods of disease prevention have resulted in scientists relying on biotechnological approaches through which ground plants can be fortified with diseases resistant genes. This will not only help the plants to counteract the disease but also benefit mankind from a nutritional and economic point of view.

Genetic Transformation: a smart alternative

Plants have the natural antimicrobial capacity and such

mechanism makes the base for understanding the major tools in combating certain pathogens like fungus. The immune mechanism, both innate and adaptive plays important role in the natural defense system of plants. In most cases, it is the unique genetic structure of a particular plant that makes it resistant to a pathogen (66). The genetic structure of the resistant varieties of the plant can be exploited through biotechnological techniques. This lead to the development of transgenic plants which are developed through genetic engineering techniques in which DNA is artificially inserted to get it expressed in recipient species (67). This advancement results in increased shelf life (61), elevated yield (68), improved nutritional quality (69), or enhancing capability of a variety to grow and yield against biotic (70-72) and abiotic stresses like tolerance to heat (73, 74), cold (76) and drought resistance (77, 78). Transformation of peanut for incorporating transgene has been started later than 1990. However, approaches targeting resistance to fungus have been initiated decades later. The next section and Table 2 have summarized the reports focused on research trends in transgenic fungus-resistant peanuts.

Research trends in transgenic fungus resistant peanuts

The application of biotechnology and genetic engineering is widely used for the development of agronomically valua-

ble qualitative and quantitative traits i.e., biotic and abiotic stress-resistant transgenic crops, which certainly helps to progress in the extension of limited gene pools in conventional breeding (79). Transgenic research offers cultivar development through the improvement of genetic resources, which could play a significant role in establishing new and useful genetic diversity and production in peanuts. However, the shortage in basic genome infrastructure, tools and resources impeded the peanut research progress than other crops e.g. potato, maize, soybean, wheat and sorghum in molecular genetics technology (80). The integration of the disease-controlling genes into the plant genome offers a steady option instead of using pesticides or bio-control agents (81). Fungal diseases mostly hampered peanuts cultivation in both the tropic and semi-arid tropic regions among all the biotic stresses (82). The first peanut transformation followed by embryogenic tissue culture was performed using the Biolistic (bombardment technique) method (83). Only 1% transformation frequency was achieved and showed stable transformed expression. They suggested the additional resting period in non-selective conditions can increase the surviving rates of transformants (83).

Some genes were incorporated through transgenic approaches to develop fungal disease-resistant peanuts (Table 2). Peanut production and quality are mainly hin-

Table 2. Selected fungal resistant transgenic peanut varieties

Peanut genotype	Transformation method	<i>Agrobacterium</i> strain	Explant	Promoter	Selectable marker	Transgene and source	Transformation efficiency (%)	Traits	Reference
TMV2	<i>Agrobacterium</i> mediated	LBA 4404	Embryo with one cotyledon	CaMV35S	<i>npt II</i>	CHI, tobacco	40	Fungal resistance against pathogen <i>Cercospora arachidicola</i> .	(99)
Okrun	Biolistic	-	Somatic embryo	CaMV35S	<i>hph</i>	Rice chitinase Alfalfa glucanase	86	Hydrolase activities of transgenic peanuts.	(81)
Okrun	Biolistic	-	Somatic embryo	CaMV35S	<i>hph</i>	Rice chitinase Alfalfa glucanase	-	Greenhouse testing of <i>Sclerotinia minor</i> resistance transgenic lines.	(96)
NC-7, Wilson, and Perry	Biolistic	-	Somatic embryo	CaMV35S	<i>hph</i>	Barley oxalate oxidase	-	Field evaluation of transgenic lines for <i>Sclerotinia minor</i> resistance.	(105)
Okrun	Biolistic	-	Somatic embryo	CaMV35S	<i>hph</i>	Rice chitinase Alfalfa glucanase	-	Fungal resistance observed under field conditions.	(79)
JL24	<i>Agrobacterium</i> -mediated	EHA 105	Embryo axes	CaMV35S	<i>npt II</i>	BjD, mustard RsAFP1 and RsAFP2	41.1	Transgenic plants showed enhanced resistance against leaf spot disease.	(108)
Georgia Green	Biolistic	-	Somatic embryo	CaMV35S	<i>hph</i>	Chloroperoxidase	86	<i>In vitro</i> and <i>situ</i> <i>A. flavus</i> inoculation.	(90)
TMV2	<i>Agrobacterium</i> mediated	LB4404	Two-day old seedlings	CaMV35S	<i>npt II</i>	uidA (β 1-3 glucanase)	40	Resistance towards <i>C. arachidicola</i> and <i>A. flavus</i> and reduced number of spots and delay onset of disease.	(91)
Wilson, Perry, NC-7	Biolistic	-	Somatic embryo	CaMV35S	<i>hph</i>	Barley oxalate oxidase	69.2	Field evaluation of transgenic lines for <i>Sclerotinia minor</i> resistance.	(45)
Golden	<i>Agrobacterium</i> -mediated	LBA 4404	Coty	CaMV35S	<i>bar</i>	<i>Bchit</i> from bacteria	-	Fungal resistance against <i>C. Arachidicola</i> .	(100)

BARI-2000, Golden	<i>Agrobacterium</i> -mediated		Cotyledonary node	CaMV35S (EN4)	<i>Hpt</i>	RCG-3	32 41	Fungal resistance against <i>C. arachidicola</i> .	(101)
JL24	<i>Agrobacterium</i> mediated	GV2260	Cotyledon	CaMV35S	<i>hpt</i>	SniOLP+rs-AFP2	65	Enhanced late leaf spot disease Resistance and reduced number and size of lesions on leaves.	(110)
Huaya 20 Huaya 22	<i>Agrobacterium</i> -mediated	EHA105	Embryonic leaflets	CaMV35 S	<i>hph</i>	β 1-3 glucanase	-	Transgenic plants showed resistance against <i>C. personata</i> .	(104)
JL-24	<i>Agrobacterium</i> -mediated	EHA105	Somatic embryo	CaMV35 S	<i>npt II</i>	AdSGT1	83.3	Peanut plant with enhanced resistance against the late leaf-spot disease.	(112)
BARD-92, BARI-2000, BARD-479, and Golden	Whisker mediated-		Epicotyl	CaMV35 S	<i>hpt</i>	EN4+RCG 3	6.88	Chitinase gene enhancing against leaf spot disease.	(113)
JL24	<i>Agrobacterium</i> mediated	C58	Embryo	CaMV35 S	None	MsDef1 and MtDef4.2 (from <i>Medicago sativa</i> and <i>M. truncatula</i> respectively)	-	Silencing two genes (<i>aflM</i> and <i>aflP</i>) to raise immunity against aflatoxin contamination.	(92)

dered by aflatoxin contamination through fungal infection (84). *Aspergillus flavus* and *A. parasiticus* produce aflatoxin, which is identified as a human carcinogen in shelled peanuts (85, 86). These highly toxic secondary metabolites are in 21% of peanuts in India, according to the Indian Council of Medical Research-Lucknow. A study performed by International Crops Research Institute for the Semi-arid Tropics (ICRISAT) showed a 40 times higher level of aflatoxin in Indian peanuts than the tolerable limits (87). A high level (423 ng g⁻¹) of aflatoxin was also found in Bangladeshi peanuts, which is much higher than the normal regulatory level in the US and European countries (88). Incorporation of *Bly1AI* gene from *Bacillus thuringiensis* was observed lessening the aflatoxin contaminations of peanut seed by *A. flavus* in transgenic peanuts. In addition, the transformation of peanut with soybean *loxI* gene showed suppression of aflatoxin biosynthetic pathway *in vitro*, and the inclusion of carrot embryo-specific promoter (DC3) in transformation showed a reduced level of aflatoxin contamination (89). Though there is suitable germplasm with less preharvest aflatoxin contamination intended for breeding in peanuts, *A. flavus* resistant transgenic peanut was also developed through genetic engineering using non-heme chloroperoxidase gene (*cpo-p*) from *Pseudomonas pyrocinia*. The embryonic tissues extract expressed 50% antifungal activity, whereas the leaf extracts of mature plants showed a 20% reduction in fungal colonies' growth. The difference could be the result of physiological or developmental changes in various plant parts. *A. flavus* (70-GFP) strain showed a 50–80% reduction in hyphal growth in the cotyledons of transgenic seeds in an *in situ* inoculation experiment (90). Aflatoxin resistivity, including a reduction in fungal hyphal spread against *A. flavus*, was also seen in a transgenic peanut obtaining PR protein glucanase, hydrolyses gene from tobacco, which acts as a plant defense barrier too against leaf spot disease caused by *Cercospora arachidicola* to delay in disease encounter with lesser leaf spots in three peanut cultivars, JL 24, ICGV 89104 and ICGV 86031 (91). The

chitinases and glucanases, both hydrolytic enzymes, can be used in developing transgenic peanuts with the capability of degradation of the fungal cell wall and spore formation. Two antifungal defensin genes (*MsDef1* and *MtDef4.2*) were transformed in peanut from *Medicago sativa* and *M. truncatula* respectively to enhance the resistance of peanut against *A. flavus* infection, and two genes of aflatoxin biosynthetic pathway (*aflM* and *aflP*) were silenced using host-induced gene silencing (HIGS) technique to reduce aflatoxin level (92). RNA interference (RNAi) mediated silencing of five aflatoxin synthesis genes, namely AFL2G_07223 (*aflS* or *aflJ*), AFL2G_07224 (*aflR*), AFL2G_07228 (*aflC/pksA/pksL1*), AFL2G_07731 (*pes1*) and AFL2G_05027 (aflatoxin efflux pump, *aflP*) controlled mycotoxin accumulation in plants, as it showed up to 100% decrease in aflatoxin contamination in peanut lines 288-72 and 288-74 (93). However, field adaptation of these transgenic plants needs more research (94).

Introduction of class II chitinase gene from rice (*Oryza sativa*) and or a glucanase from alfalfa (*M. sativa*) into peanut resulted in transgenic peanut lines production with 25% higher chitinase and glucanase activities. Many varieties, including peanuts, produce chitinase, observed in non-transformed plants; however, more analysis is required to confirm the glucanase activity as there is no endogenous β -1-3-glucanase gene reported to be found in peanut (81). Many fungi are susceptible to the combination of chitinase and β -1,3-glucanase than chitinase alone (95). The cultivar Okrun, known for its desirable traits and better tissue culture performance, presented as the parent genotype to all transgenic lines. These peanut lines were investigated for their stable transgene expression (81), also for their reaction to fungal contamination in the greenhouse (96) and field conditions (79). Chitinases show a different level of antifungal activity against a wide range of fungal strains under *in vitro* conditions; however, the protection level is influenced by several factors, i.e. enzyme activity

and expression, protein concentration, pathogen characteristics, host-pathogen interaction (97). The overexpression of Chitinase activity significantly decreased fungal infection frequency in various plants by slowing down fungal growth and inducing plant defense mechanisms (98). The next generation of transgenic peanuts containing chitinase genes (*CHI* and *Rchit* and from tobacco and rice respectively, and bacterial *Bchit*) was tested for their resistance to *Fusarium* wilt and leaf spot or tikka disease (*C. arachidicola*) (99-101). Transformation with bacterial chitinase (*Bchit*) and rice chitinase (*RCG-3*) genes showed higher enzyme activity but the resistance intensity varied against *C. arachidicola* (100, 101). The variation in pathogen resistance could be due to the determination of chitinase enzymes at the tissue and cellular levels (102, 103). Two to fourteen-fold higher chitinase expressions were observed in peanuts against *A. flavus* when transformed with rice chitinase gene (*Rchit* gene), and 25% less infection was also observed in seed colonization assay performed *in vitro*. A negative correlation between disease frequency and chitinase activity was identified as fewer lesions were observed from leaf spot (LLS) and rust diseases (98). Peanut plants expressed the β -1,3-glucanase gene, confirming an improved disease resistance against *Cercospora personata* (104).

Expression of barley oxalate oxidase gene in a transgenic peanut developed an increased resistance against *Sclerotinia* blight caused by necrotrophic fungi *Sclerotinia minor*. The connection of pathogenic reaction between oxalic acid and *Sclerotinia* species was demonstrated in a study. cDNA sequence of barley oxalate oxidase was used for protein expression, which degrades the oxalic acid content to develop transgenic varieties resistant to *Sclerotinia* blight. The lesion size was reduced to 75% to 97% compared to the non-transformed plants (105). These transgenic plants were observed a significant reduction in disease with an increased yield ranging from 488–2755 kg/ha compared with non-transgenic lines over 5 years (45).

Plant-based pathogenesis-related (PR) proteins are induced by plants as a defense response and express toxicity towards the disease-causing fungal pathogens (106). The PR proteins are classified into 17 families according to their mode of action and some can enzymatically hydrolyze the fungal cell walls (107). Pathogenesis-related proteins and defensins are useful antifungal proteins and can potentially achieve biosafety approval for commercial use due to their endogenous production capability in plants. Identification of these proteins in plants unlocks a new possibility for establishing disease-resistant transgenic plants through overexpression. Defensin gene (*BjD*) from mustard (*RsAFP-1* and *RsAFP-2* of *Raphanus sativus*) was transferred in transgenic peanuts exhibiting better resistance to severe late leaf spots caused by both *Pheoisariopsis personata* and *C. arachidicola* (108). Leaf spot disease has one of the most devastating effects on crop yield as 30-48% yield loss was observed by the early and late leaf spot in peanuts in Bangladesh (109). Reduction in lesion size and the onset of late leaf spot disease (caused by *P. personata*) were observed in a transgenic peanut plant expressing a combination of PR genes *SniOLP* (*Solanum nigrum* osmotin-like protein) and *Rs*

-AFP2 (*R. sativus* antifungal protein-2) in an experiment performed both at laboratory and greenhouse conditions (110).

Several genes from wild peanut *A. diogeni* have been characterized, which are involved in plants' defense, and showed resistance against the late leaf spot pathogen (111). Transgenic peanut plants obtained *AdSgt1* through in planta transformation showed improved resistance to the late leaf spot pathogen and the expression induced the emergence of resistance-related genes, CC-NB-LRR, and some protein kinases (112). A transformation was done using silicon carbide whiskers to obtain resistance against leaf spots by transferring the chitinase gene. The highest transformation efficiency (6.88%) was obtained from 20 mg of two-day-old callus using 200 mg of whiskers with a five μ g plasmid. This time-saving and cost-effective procedure can successfully introduce transgene directly into legumes and can avoid overexpression and inheritance problems (113).

Peanut kernel production of stilbene phytoalexins, which is induced by fungal infections, hinders fungal growth and spore formation. Resveratrol (an antioxidant) production was observed in tobacco transformed with stilbene synthase isolated from peanuts (114). Peanut is considered as a recalcitrant crop to perform *in vitro* regeneration and transformation; however, a high-frequency transformation rate was achieved using cotyledonary and embryo axes explants. Several transgenic lines were successfully established, but none of them has been released till now. Besides, public resistance and expensive regulatory requirements negatively impact the process (84).

Challenges in Peanut Transformation

Introducing novel antifungal genes into peanuts is preferable over conventional plant breeding systems and it is expected to limit the use of commercial fungicidal agents for managing fungal attacks which is the foremost reason for production loss in peanuts worldwide. Transformation allows introgression of a gene(s) that is not common in the *Arachis* genus or may bear a pleiotropic role for peanut production i.e., peanut yield, flavor, quality etc. Though approaches for efficient transformation systems have been initiated 30 years ago, regrettably transformation and regeneration are still facing several challenges for peanut and other valuable crops (115, 116).

For developing fungus-resistant peanut and other legumes, researchers are working for a reliable protocol that is efficient, reproducible, genotype and location independent, also takes a shorter time for a variety. Besides, transformation strategies focus on components like,

- A potential source of candidate genes.
- Prospective assortment of totipotent explants like cotyledon, leaves, gametes etc.
- The appropriate DNA delivery method into the target cell.
- Selection of transformed cells in various phases of plant development.

Moreover, transgenic plants should not possess aspects like insertional mutagenesis, pleiotropy, and

somaclonal variation which impedes the necessary agronomic characteristics (117). In this context, several issues are required to point a light over them for addressing peanut transformation.

Tissue culture vs. Non-tissue culture-based transformation: While designing a transformation strategy, the first query comes on whether the method will be tissue culture-dependent or not? Different tissues of peanut namely, leaflet, somatic embryo, embryo axis, cotyledon and hypocotyl have been successfully used as explants in generating transgenic lines (118). Most of the peanut transformation reported on tissue culture-dependent method but there are reports on other legumes of following non-tissue culture-dependent method of transformation. For example, the meristem multiplication method described (119) for soybean, *Agrobacterium tumefaciens* based method for a peanut (99) were successful to bypass the tissue culture phase (120). The tissue culture-based method often confronts the difficulty of recalcitrancy of peanut tissues. After transformation, peanut regeneration often shows a poor response and low frequency of transformed plants. It also requires professional skill to maintain the varieties amenable to regeneration (120). Besides, tissue necrosis (browning of putatively transformed tissue) is another constraint faced on the tissue culture-dependent method. During co-cultivation of *Agrobacterium* and plant cells, browning of tissues has been observed to have resulted from elevated peroxidase activity of plant-bacterium interaction (121). Crops like grape (122), sugarcane (123), rice (124) etc. have been reported to overcome tissue necrosis when culture media was supplemented with antioxidants, but there is no report of peanut or other legumes to achieve such (121).

Chimera: A failure to transform gene stability: Recent advances in molecular biology techniques have made a list of genetic tools available for the isolation of potential candidate gene(s) from competent species and transform them into a peanut. But there is a gap in gene stability in subsequent generations of putatively transformed peanut (84). This lacuna is the reason for the generation of chimeric plants in legumes, which has been reported for chickpea, lentils (116, 125, 126). After transformation, when a plant tissue escapes transmission of genes in next-generation or develops as a combination of transformed and non-transformed portions, these plant lines are denoted as chimeric lines. The occurrence of chimera diminishes the efficiency of the transformation system and thus limits the scope of generating a stable transgenic line (116). Chimeric expression of the "GUS" reporter gene has been reported for peanuts, but a chimeric expression of the candidate gene has not been observed yet (92). Constant observation is still needed for achieving a stable fungal-resistant transgenic line in peanuts.

Controlling transgene expression: Improvement of transformation frequency, accompanied by identification of novel promoter and enhancer elements, isolation of functional genes denoted as crucial factors in transgenic plant development. In addition, control of temporal and spatial expression of an exogenous anti-fungal gene(s) is critical for achieving efficacious transformation (127).

Hence, promoters provide foundational monitoring of gene expression in forms like constitutive, inducible, tissue-specific, developmental etc. Though successful promoter-less transformation has been reported and still most researchers rely on regulatory control mediated by promoters (128).

Earlier approaches of peanut transformation reported the use of constitutive promoters namely CaMV35S or potato ubiquitin 3 promoters, which were observed to be active in the whole plant and failed to protect the edible portions of the respective plant from the generation of potentially harmful byproducts (127, 129, 130). Besides, these promoters have been noticed to increase energy demand on host plants which leads to reduced plant growth and yield. Also, continuous exposure of the toxic gene to the fungal agents may enhance the probability of peanut developing resistance (127). In addition, plants transformed with plasmids featuring constitutive promoters have been reported to experience homology-dependent transcriptional silencing (131). Such adverse ectopic expression can be avoided by the inducible or tissue-specific promoter, which delivers regulated control of transgene expression (127). Seed specific promoters i.e., cottonseed α -globulin B gene promoter (132), barley lemma gene promoter (*lem1*) (133) etc. have been reported for seed-specific expression of the anti-fungal gene in crop seeds, which have shown a new way for peanut transformation. Seed-specific promoters have been isolated and characterized in peanuts (134). Also, seed-specific expression of the *AtLEC1* gene has been reported to increase oil content and lipid reservoir in peanuts (135). However, seed-specific expression of an anti-fungal gene in peanuts through transformation is about to be explored.

Subsequently, pathogen/wound inducible promoters such as maize proteinase inhibitor gene promoter (*mp/*) (136), poplar *win3.12T* (137) gene promoter can be other choices for controlling fungal invasion at the place of infection which may reduce the probability of any lethal effects on plant growth and development (127). However, the transformation of transgene downstream to a promoter may interfere with the host plant's normal gene function by modifying transcriptional control of upstream or downstream plant promoter which may lead to disruption of other gene functions (138). In such a situation, a transformation of promoter-less construct can be carried out in peanuts, where the anti-fungal gene may be regulated by the upstream plant regulatory sequence that has been hooked with it (108). Besides, the synthetic promoter can be investigated for expressing functionally important genes in transgenic plant research (139).

Perspectives on environmental issues: Development of a fungus-resistant peanut or some other genetically modified legume cultivar will be required to address several issues during field trials concerning public acceptance. These issues include:

- The effect of spreading the transgene to related species and its impact on ecology.
- The possible non-targeted side-effects on other or-

ganisms like beneficial microorganisms, insects, rodents etc.

- Health effects of over-expression of the transgene on edible plant parts.
- Possibility of achieving resistance of the existing pathogens or evolving to new pathogen strains.
- Presence or absence of allergic elements in the transformed line etc.

Though any adverse effects have not been reported for legume transformation, these issues are required to monitor regularly (140).

Issues regarding peanut transformation and developing cultivar resistance to fungal agents should look for scopes to improve transformation technology while proper monitoring is also required. The advancement of plant biotechnology techniques can act as a ray of hope in this regard.

Prospects regarding peanut transformation

Introducing a novel trait to a plant and releasing it as a new variety relies on continuous improvement to reach the goal. There are still hurdles that need to solve by using molecular biology tools and other plants' biotechnological knowledge.

Improvisation of transformation techniques: Many reports have been mentioned about inefficient DNA delivery by *Agrobacterium*, which is being tried to overcome by recent developments of super-binary vectors and highly virulent strains (141, 142). Also, analysis of host-pathogen interaction should be followed for characterization of peanut genotypes and *Agrobacterium* strains which may be compatible partners for transformation and are expected to improve transformation efficacy (143). Chemical compounds like acetosyringone (AS) and thiols are supplemented individually in a co-cultivation medium, have been reported to significantly increase the number of transformed plantlets in rice (144) and peanuts (145). Acetosyringone stimulates vir gene in the Ti plasmid of *Agrobacterium* which enhances the scope of infection to explant (142). Thiol compounds have been reported to inhibit peroxidase and polyphenol oxidase in explants during cocultivation which facilitates T-DNA delivery to explants (146). There is scope to improve more peanut cultivars and other legumes transformation by using these compounds. Measures like explant wounding /micro-injury have been widely followed in transformation studies where some of them have reported a low frequency of transient transformation (116, 147). As an alternative to mechanical wounding, sonication-induced tissue wounding or whisker-mediated transformation can be tried to increase the transformation frequency (113, 146). SAAT implies alternating exposure of explants to sonication waves in presence of *Agrobacterium* which can overcome the cell-wall barrier of explants for efficient transformation (148). Also, transformation using whiskers has advantages like micro-puncturing of cells for DNA delivery through penetration of cell wall which reaches to the nucleus directly and technical expertise is not required like gene-gun method (113). Besides nuclear transformation,

new insight toward plastid transformation can be explored as this technique offers distinctive features like the prohibition of the outcrossing of transgene and recombinant products, stable production of recombinant protein, excludes gene silencing and position effects and cellular compartmentalization of compounds unfavorable to the plant (149, 150).

Characterization of novel anti-fungal gene: Besides improving the transformation strategies, finding the appropriate anti-fungal gene is the critical step needed to achieve fungal disease resistance in peanuts. The access to cDNA sequences has been made intense understanding of gene characteristics, development of markers, constructing microarray and genome annotation (151-153). It has been a decade that, microarray has been proposed to study the expression pattern of defense reaction in peanut plants (154). Since then, Expressed Sequenced Tags (ESTs) have been used to detect key genes involved in defense response in peanut against fungal infections and aspired to uncover gene expression and regulation (155-157). The interaction of peanut ESTs and the fungus *Cercosporidium personatum* (causing late leaf spot) has been reported using suppressive subtractive hybridization (SSH) technique to build up cDNA library has made way for further research in peanut transformation (158). Table 3 illustrates some selected reports which have been published using ESTs for understanding molecular features behind fungal resistance in peanuts.

Utilizing Marker-assisted selection, previously reported stress-resistant genes can be tried to enhance resistance against fungal infection in peanuts by introducing them through transformation (164, 165). Furthermore, quantitative trait loci mapping (QTLs) has revealed regions in the peanut chromosome for resistance against early and late leaf spots and stem rot infection through marker-assisted selection. These observations have validated the potency of the gene pyramiding approach to achieve resistance against fungal attack and the development of a superior cultivar to limit the disease spread and minimize

Table 3. ESTs for characterization of anti-fungal genes from different fungal species in peanut

Fungus strain	Diseases	Technique	References
<i>Cercospora arachidicola</i>	Early leaf spot fungi	RNA-Seq analysis EST-SSR markers	(159)
<i>Sclerotium rolfsii</i>	Stem rot	RNA-Seq analysis	(160, 161)
<i>Aspergillus flavus</i> <i>Aspergillus parasiticus</i>	Postharvest rot	quantitative trait loci mapping (QTLs)	(162)
<i>Metarhizium anisopliae</i>	Beneficial fungi	transcriptome sequencing	(163)
<i>Fusarium oxysporum</i>	Root rot pathogen	cDNA-AFLP	
<i>Phaeoisariopsis personata</i>	Late Leaf Spot	2D proteomics ESTs	(111)

yield loss (10, 166). In addition, antifungal proteins like *Aleuria aurantia* lectin (against *Mucor racemosus*), *Coprinosin* lectin 2 (from *Ashbya gossypii*) (167), lipid transfer protein (against *A. flavus*) (75); lectin from *Spar-*

assis latifolia against *Candida* and *Fusarium* sp. (168) etc. have been examined by the gene-pyramiding approach to achieve strong bioactivity against fungal agents. Genetic transformation of such genes is expected to enhance fungal resistance in peanuts.

RNAi gene silencing: RNA interference (RNAi) gene silencing is a unique mechanism of gene regulation mediated by small RNAs (sRNA) acting on a key gene(s) to silent its expression at the transcriptional or post-transcriptional level thereby limiting fungal growth (127). It was reported that RNAi silencing for resistance-associated proteins (RAPs) to achieve aflatoxin resistance in maize (127, 169). For peanuts, a reduced colonization of *A. flavus* and aflatoxin contamination in the kernels has been reported by reducing organ-specific expression of stilbene synthase through RNAi silencing (170). This silencing technique targeted to a variety of fungal agents (in peanut and other plants) has been listed up in Table 4. Different varieties of peanut can be tested to adopt this technology for improving peanut transformation in near future.

Coevolution of Host-Pathogen Interaction: Plant-*Agrobacterium* relation delineates the possibilities of successful genetic transformation. Systematic observation of defense response gene transcripts has revealed the intricate relationship between pathogen invasion and host plant response (113, 143). Fungal pathogens may obtain resistance to the engineered traits through natural selection, adaptivity, environmental factors etc. These genetic changes in the attacking pathogens take place parallel to the engineered plant, which is denoted as co-evolution (140). The fungal attack has been noted for adaptive func-

tion and plant biotechnology, new approaches should be tested for better results and the improvisation of present transformation techniques. But it should be kept in mind that, along with new approaches, there will be new problems to solve. Therefore, a constant intensification of research is necessary to be carried out in the future.

Future prospects and conclusion

The fungal diseases of peanuts have been a matter of great concern in plant science and agriculture, considering a massive amount of annual yield losses incurred due to infestation by fungi. A variety of traditional and commercially available measures are popular among farmers, which are being protested by green activists for diversified reasons. Hence, disregarding the conventional approaches, there is a thrust in biotechnological approaches, which are more environment friendly for combating peanut disease resistance and are presumed to have the non-existence of deleterious effect on the ecosystem. In this regard, peanut has been considered as a reliable candidate for the transformation of candidate genes, to achieve economic benefits during its farming. Numerous attempts have been tried to confer fungal resistance in peanuts using different ex-plants, techniques, conditions, though commercially available genetically modified fungus-resistant peanut is yet to be available. Still, most of the research outcomes are based on laboratory environment, which makes a growing demand on long-term field testing of the candidate fungal-resistant peanut varieties. Some other factors needed to be checked before the field trial. Firstly, achieving fungal-disease resistance is highly complex because it engages multigenic features. It is worth noting that, incorporation of a single gene may provide insufficient resistance while the incorporation of multiple genes may pose a burden on the plant. Proper coordination is highly recommended to achieve an acceptable level of resistance while keeping the physiological features and yield uninterrupted. Next, Epigenetic modifications are necessary to be addressed to control transgene stability in transgenic peanuts. As it is hypothetical to monitor the integration of a gene (or the number of its copies) into the recipient organism; this makes transgene regulation more difficult. It may lead to epigenetic silencing of the candidate transgene and foresee its effect on transgenic peanuts. Besides, testing in a local environment is required to check the ecological relevance and agronomic performance. The concerning issues for biosafety regulation need to be addressed during the trials to attract public acceptance and farmers' interest.

The exploration of genes from wild relatives of *Arachis* has also been undertaken with a motive to fortify peanuts from fungal disease. Variance regarding transformation efficiency has not been observed in various techniques studied so far; however, there are specific challenges in implementing the biotechnological method for the development of disease resistance peanut plants, i.e., the tissue culture is often associated with low recalcitrance. The low transformation frequency and the browning process resulting from peroxidase activity have also been reported, whereas hindrances like tissue browning and transgene instability are yet to address. Therefore, intense

Table 4. Examples of using RNAi silencing to inhibit fungal species in peanut

Plant	Fungal strain	Disease	References
Peanut	<i>Aspergillus flavus</i>	Post-harvest loss	(171)
Peanut	<i>Aspergillus flavus</i>	Control of Aflatoxin production.	(93)
Peanut	<i>Aspergillus flavus</i>	Control of Aflatoxin production	(170)
Barley	<i>Fusarium graminearum</i>	<i>Fusarium</i> head blight	(172)
Soybean	<i>Macrophomina phaseolina</i>	Charcoal rot disease	(173)
Grapevine	<i>Botrytis cinerea</i>	Grape bunch rot	(174)
Mustard	<i>Scclerotium sclerotiorum</i>	Foliar application	(175)
	<i>Botrytis cinerea</i>		
Wheat	<i>Fusarium asiaticum</i>	Wound	(176)
Bean	<i>Uromyces appendiculatus</i>	Rust	(177)
Potato	<i>Phytophthora infestans</i>	late blight	(178)

tional modifications of the enzyme chitinase active site in tobacco and rice (95). Likewise, pathogen sensitivity to the anti-fungal protein over-expressed in a transgenic plant can be modified by adaptivity. The possibility of co-evolution is a matter of concern from the perspective of developing the transgenic plant as well as environmental issues (94). Regrettably, this issue is often unnoticed by researchers. Future research in peanut transformation should handle it methodologically. With the advancement of molecular biol-

investigations and improvised techniques are a need of time to make the process of genetic methods of transformation more effective and successful. It will not only help in building genetically stabilized peanut lines to combat fungal infection effectively but also can consequently minimize the yield loss and optimize productivity.

On a separate front, aflatoxin contamination in peanuts also raises apprehension as it is likely to impart its toxicity to the population. Devising more efficient means to control *Aspergillus* infection and develop the resistant traits can be consequently added to the nutrition of hunger-stricken populations throughout the globe. Thus, biotechnological approaches centered on genetic transformation are the preferably effective strategy in combating the fungal disease of peanut and are of extreme relevance, especially in the countries of the Indian subcontinent, for increased productivity and better nutritional benefits. Possibly peanut transformation will serve as a new platform to discover the unrecovered aspects of epigenetics.

Acknowledgements

The authors are thankful to the libraries of their respective Institutions for providing the online platform for the literature survey. The authors are also thankful to National Library, Kolkata and American Centre Library, Kolkata for providing online infrastructural facilities to search relevant research papers and articles required for framing the article.

Authors contributions

All authors have contributed equally in composing the manuscript.

Compliance with ethical standards

Conflict of interest: None.

Ethical issues: None.

References

- Otyama PI, Kulkarni R, Chamberlin K, Ozias-Akins P, Chu Y, Lincoln LM, MacDonald GE, Anglin NL, Dash S, Bertioli DJ, Fernández-Baca D, Graham MA, Cannon SB, Cannon EKS. Genotypic Characterization of the U.S. Peanut Core Collection. *G3 (Bethesda)*. 2020;10(11):4013-26. <https://doi.org/10.1534/g3.120.401306>.
- Wright G. Peanuts. In: Wrigley G, editor. *Encyclopedia of Grain Science*. Elsevier; 2004. p. 438-44. <https://doi.org/10.1016/B0-12-765490-9/00125-7>.
- Prasad PV, Kakani VG, Upadhyaya HD. Soils, Plant Growth And Crop Production. 2nd ed, Vol II. Oxford, U.K.: In EOLSS Publishers; 2010. p.1-34.
- Arya SS, Salve AR, Chauhan S. Peanuts As Functional Food: A Review. *J Food Sci Technol*. 2016;53(1):31-41. <https://doi.org/10.1007/s13197-015-2007-9>.
- Senakoon W, Nuchadomrong S, Chiou RY-Y, Senawong G, Jogloy S, Songsri P et al. Identification of peanut seed prolamins with an antifungal role by 2D-GE and drought treatment. *Biosci Biotechnol Biochem*. 2015;79(11):1771-8. <https://doi.org/10.1080/09168451.2015.1056508>.
- Pal KK, Dey R, Tilak KVBR. Fungal Diseases of Groundnut: Control and Future Challenges. In: Goyal A., Manoharachary C, editors. *Future Challenges in Crop Protection Against Fungal Pathogens*. Fungal Biology. Springer, New York; 2014. p. 1-29. https://doi.org/10.1007/978-1-4939-1188-2_1.
- Iquebal MA, Tomar RS, Parakhia M V, Singla D, Jaiswal S, Rathod VM et al. Draft whole genome sequence of groundnut stem rot fungus *Athelia rolfsii* revealing genetic architect of its pathogenicity and virulence. *Sci Rep*. 2017;7:5299:1-10. <https://doi.org/10.1038/s41598-017-05478-8>.
- Olatinwo RO, Prabha TV, Paz JO, Hoogenboom G. Predicting favorable conditions for early leaf spot of peanut using output from the weather research and forecasting (WRF) Model. *Int J Biometeorol*. 2012;56(2):259-68. <https://doi.org/10.1007/s00484-011-0425-6>.
- Xu ML, Yang JG, Wu JX, Chi YC, Xie LH. First report of *Aspergillus niger* causing root rot of peanut in China. *Plant Dis*. 2015;99(2):284-84. <https://doi.org/10.1094/PDIS-05-14-0530-PDN>.
- Chu Y, Chee P, Culbreath A, Isleib TG, Holbrook CC, Ozias-Akins P. Major QTLs for resistance to early and late leaf spot diseases are identified on chromosomes 3 and 5 in peanut (*Arachis hypogaea*). *Front Plant Sci*. 2019;10:883:1-13. <https://doi.org/10.3389/fpls.2019.00883>.
- Sobolev V, Walk T, Arias R, Massa A, Lamb M. Inhibition of aflatoxin formation in *Aspergillus* sp. by peanut (*Arachis hypogaea*) seed stilbenoids in the course of peanut-fungus interaction. *J Agric Food Chem*. 2019;67(22):6212-21. <https://doi.org/10.1021/acs.jafc.9b01969>.
- Vargas Gil S, Haro R, Oddino C, Kearney M, Zuza M, Marinelli A et al. Crop management practices in the control of peanut diseases caused by soilborne fungi. *Crop Prot*. 2008;27(1):1-9. <https://doi.org/10.1016/j.cropro.2007.03.010>.
- Zubrod JP, Bundschuh M, Arts G, Brühl CA, Imfeld G, Knäbel A et al. Fungicides: An overlooked pesticide class? *Environ Sci Technol*. 2019;53(7):3347-65. <https://doi.org/10.1021/acs.est.8b04392>.
- Hahn M. The rising threat of fungicide resistance in plant pathogenic fungi: *Botrytis* as a case study. *J Chem Biol*. 2014;7(4):133-41. <https://doi.org/10.1007/s12154-014-0113-1>.
- Panth M, Hassler SC, Baysal-Gurel F. Methods for management of soilborne diseases in crop production. *Agriculture*. 2020;10(1):16. <https://doi.org/10.3390/agriculture10010016>.
- Vincelli P. Genetic engineering and sustainable crop disease management: Opportunities for case-by-case decision-making. *Sustainability*. 2016;8(5):495. <https://doi.org/10.3390/su8050495>.
- Rathnakumar AL, Singh R, Parmar DL, Misra JB. Groundnut: A crop profile and compendium of notified varieties of India. 2013 [Cited 2021, September 1]. Directorate of Groundnut Research, Junagadh, Gujarat, India. 118p. Available from: <https://krishi.icar.gov.in/jspui/handle/123456789/4453>.
- Kumar R, Pandey MK, Roychoudhry S, Nayyar H, Kepinski S, Varshney RK. Peg biology: Deciphering the molecular regulations involved during peanut peg development. *Front Plant Sci*. 2019;10:1289:1-17. <https://doi.org/10.3389/fpls.2019.01289>.
- Ghewande MP, Desai S BM. Diagnosis and management of major diseases of groundnut. *NRCG Bull*. 2002;8-9.
- Pande S, Bandyopadhyay R, Blümmel M, Narayana Rao J, Thomas D, Navi S. Disease management factors influencing yield and quality of sorghum and groundnut crop Residues. *Field Crops Res*. 2003;84(1-2):89-103. [https://doi.org/10.1016/S0378-4290\(03\)00143-6](https://doi.org/10.1016/S0378-4290(03)00143-6).

21. Moradia AM, Khandar RR. Loss of yield of groundnut (*Arachis hypogaea* L.) due to dry root rot (*Macrophomina phaseolina*) and their management under *in vivo* condition. *Int J Agric Sci.* 2011;7(2):282–85.
22. Rani PU, Yasur J. Physiological changes in groundnut plants induced by pathogenic infection of *Cercosporidium personatum* Deighton. *Allelopathy J.* 2009;23(2):369–78.
23. Nutsugah SK, Abudulai M, Oti-Boateng C, Brandenburg RL, Jordan DL. Management of leaf spot diseases of peanut with fungicides and local detergents in Ghana. *Plant Pathol J.* 2007; 6(3):248–53. <https://doi.org/10.3923/ppj.2007.248.253>.
24. Hollowell JE, Shew BB, Beute MK, Abad ZG. Occurrence of pod rot pathogens in peanuts grown in North Carolina. *Plant Disease.* 1998;82(12):1345–49. <https://doi.org/10.1094/PDIS.1998.82.12.1345>.
25. Beute MK. Pythium diseases. In: Kokalis-Burelle N, Porter DM, Rodriguez-Kabana R, Smith DH, Subhrahmanyam P, editors. *Compendium of peanut diseases.* 2nd ed. St. Paul, Minn, USA:APS Press;1997. p. 27–30.
26. Anco DJ, Hiers JB, Thomas JS. Improved management efficacy of late leaf spot on peanut through combined application of prothioconazole with fluxapyroxad and pyraclostrobin. *Agronomy.* 2020;10(2):298. <https://doi.org/10.3390/agronomy10020298>.
27. Subrahmanyam P, McDonald D. Rust disease of groundnut. *Information Bulletin, No. 13, ICRISAT, India.* 1983.
28. Chaudhari S, Khare D, Patil SC, Sundravada S, Variath MT, Sudini HK et al. Genotype environment studies on resistance to late leaf spot and rust in genomic selection training population of peanut (*Arachis hypogaea* L.). *Front Plant Sci.* 2019;10. <https://doi.org/10.3389/fpls.2019.01338>.
29. Deepthi KC, Reddy NE. Stem rot disease of groundnut (*Arachis hypogaea* L.) induced by *Sclerotium rolfsii* and its management. *Int J Life Sci Biotechnol Pharma Res.* 2013; 2(3):26–38.
30. Butzler TM, Bailey J, Beute MK. Integrated management of *Sclerotinia* blight in peanut: Utilizing canopy morphology, mechanical pruning and fungicide timing. *Plant Dis.* 1998; 82(12):1312–18. <https://doi.org/10.1094/PDIS.1998.82.12.1312>.
31. Bera SK, Kasundra SV, Kamdar JH, BC A, Lal C, Thirumalasy PP, Dash P, Maurya AK. Variable response of interspecific breeding lines of groundnut to *Sclerotium rolfsii* infection under field and laboratory conditions. *Electron J Plant Breed.* 2014;5(1):22–29.
32. Thiessen LD, Woodward JE. Diseases of peanut caused by soil-borne pathogens in the Southwestern United States. *ISRN Agron.* 2012;2012:1–9. <https://doi.org/10.5402/2012/517905>.
33. Rushing BR, Selim MI. Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure and detoxification methods. *Food Chem Toxicol.* 2019; 124:81–100. <https://doi.org/10.1016/j.fct.2018.11.047>.
34. Mohammed A, Chala A, Dejene M, Fininsa C, Hoisington DA, Sobolev VS et al. *Aspergillus* and aflatoxin in groundnut (*Arachis hypogaea* L.) and groundnut cake in Eastern Ethiopia. *Food Addit Contam Part B Surveill.* 2016;9(4):290–98. <https://doi.org/10.1080/19393210.2016.1216468>.
35. Kachapulula PW, Akello J, Bandyopadhyay R, Cotty PJ. *Aspergillus* section Flavi community structure in Zambia influences aflatoxin contamination of maize and groundnut. *Int J Food Microbiol.* 2017;261:49–56. <https://doi.org/10.1016/j.ijfoodmicro.2017.08.014>.
36. Pandey MK, Kumar R, Pandey AK, Soni P, Gangurde SS, Sudini HK et al. Mitigating aflatoxin contamination in groundnut through a combination of genetic resistance and post-harvest management practices. *Toxins (Basel).* 2019;11(6):315. <https://doi.org/10.3390/toxins11060315>.
37. N'dede CB, Jolly CM, Vodouhe SD, Jolly PE. Economic risks of aflatoxin contamination in marketing of peanut in Benin. *Econ Res Int.* 2012;2012:1–12. <https://doi.org/10.1155/2012/230638>.
38. Santra HK, Banerjee D. Fungal endophytes: A source for biological control agents. In: Yadav A, Mishra S, Kour D, Yadav N, Kumar A editors. *Agriculturally important fungi for sustainable agriculture.* Fungal Biology. Cham: Springer;2020. p. 181–216. https://doi.org/10.1007/978-3-030-48474-3_6.
39. Wallwork H. The use of host plant resistance in disease control. In: Walters D editor. *Disease control in crops: Biological and environmentally-friendly approaches.* Oxford, UK: Wiley-Blackwell;2009. p. 122–41. <https://doi.org/10.1002/9781444312157.ch6>.
40. Gorbet DW, Kucharek TA, Shokes FM, Brenneman TB. Field evaluations of peanut germplasm for resistance to stem rot caused by *Sclerotium rolfsii*. *Peanut Sci.* 2004; 31(2):91–95. <https://doi.org/10.3146/pnut.31.2.0006>.
41. Bennett RS. Growth chamber assay for evaluating resistance to *Athelia rolfsii*. *Peanut Sci.* 2020;47(1):25–32. <https://doi.org/10.3146/PS19-12.1>.
42. Morton BR, Tillman BL, Gorbet DW, Boote KJ. Impact of seed storage environment on field emergence of peanut (*Arachis hypogaea* L.) cultivars. *Peanut Sci.* 2008;35(2):108–15. <https://doi.org/10.3146/PS07-111.1>.
43. Brenneman TB. *Rhizoctonia* diseases. In: Kokalis-Burelle N, Porter DM, Rodriguez-Kabana R, Smith DH, Subhrahmanyam P, editors. *Compendium of peanut diseases.* 2nd ed. St. Paul, Minn, USA:APS Press;1997. p. 30–31.
44. Woodward JE, Brenneman TB, Kemerait Jr RC, Culbreath AK, Clark JR. *Sclerotinia* blight in Georgia and evidence for resistance to *Sclerotinia sclerotiorum* in runner peanuts. *Plant Heal Prog.* 2006;7:1–19. <https://doi.org/10.1094/PHP-2006-0531-01-RS>.
45. Partridge-Telenko DE, Hu J, Livingstone DM, Shew BB, Phipps PM, Grabau EA. *Sclerotinia* blight resistance in Virginia-Type peanut transformed with a barley oxalate oxidase gene. *Phytopathology®.* 2011;101(7):786–93. <https://doi.org/10.1094/PHYTO-10-10-0266>.
46. Woodward JE, Baughman TA, Baring MR, Simpson CE. Comparison of three high-oleic peanut cultivars under varying field conditions in the Southwestern United States. *Peanut Sci.* 2015;42(1):11–17. <https://doi.org/10.3146/0095-3679-42.1.11>.
47. Dordas C. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agron Sustain Dev.* 2008;28(1):33–46. <https://doi.org/10.1051/agro:2007051>.
48. Pérez-Montaño F, Alías-Villegas C, Bellogín RA, del Cerro P, Espuny MR, Jiménez-Guerrero I et al. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiol Res.* 2014;169(5–6):325–36. <https://doi.org/10.1016/j.micres.2013.09.011>.
49. Arif I, Batool M, Schenk PM. Plant microbiome engineering: Expected benefits for improved crop growth and resilience. *Trends Biotechnol.* 2020;38(12):1385–96. <https://doi.org/10.1016/j.tibtech.2020.04.015>.
50. Blanco NHM, Barbosa DFR, Graichen FAS. Antagonistic microorganisms and nitrogen fertilization in control of tomato southern blight. *Arq Inst Biol (Sao Paulo).* 2021;88. <https://doi.org/10.1590/1808-1657000502019>.
51. Erper I, Turkkan M, Karaca GH, Kilic G. Evaluation of *in vitro* antifungal activity of potassium bicarbonate on *Rhizoctonia solani* AG 4 HG-I, *Sclerotinia sclerotiorum* and *Trichoderma* sp. *African J Biotechnol.* 2011;10(43):8605–12. <https://doi.org/10.5897/AJB11.411>
52. Grichar WJ, Besler BA, Melouk HA. Peanut (*Arachis hypogaea*) response to agricultural and power plant by-product calcium.

- Peanut Sci. 2004;31(2):95–101. <https://doi.org/10.3146/pnut.31.2.0007>.
53. Gilbert-López B, García-Reyes JF, Molina-Díaz A. Sample treatment and determination of pesticide residues in fatty vegetable matrices: A review. *Talanta*. 2009;79(2):109–28. <https://doi.org/10.1016/j.talanta.2009.04.022>.
 54. Subash SP, Chand P, Pavithra S, Balaji SJ, Pal S. Pesticide use in Indian Agriculture: Trends, Market Structure and Policy Issues. Icar – National Institute of Agricultural Economics And Policy Research, New Delhi; 2018.
 55. Grichar WJ, Woodward JE. Fungicides and application timing for control of early leafspot, southern blight and *Sclerotinia* blight of peanut. *Int J Agron*. 2016;1–7. <https://doi.org/10.1155/2016/1848723>.
 56. Augusto J, Breneman TB, Culbreath AK, Sumner P. Night spraying peanut fungicides I. extended fungicide residual and integrated disease management. *Plant Dis*. 2010;94(6):676–82. <https://doi.org/10.1094/PDIS-94-6-0676>.
 57. Johnson RC, Cantonwine EG. Post-infection activities of fungicides against *Cercospora arachidicola* of peanut (*Arachis hypogaea*). *Pest Manag Sci*. 2014;70(8):1202–26. <https://doi.org/10.1002/ps.3671>.
 58. Yu D, Li J, Zhang Y, Wang H, Guo B, Zheng L. Enantioselective bioaccumulation of tebuconazole in earthworm *Eisenia fetida*. *J Environ Sci*. 2012;24(12):2198–204. [https://doi.org/10.1016/S1001-0742\(11\)61053-X](https://doi.org/10.1016/S1001-0742(11)61053-X).
 59. Dawoud M, Bundschuh M, Goedkoop W, McKie BG. Interactive effects of an insecticide and a fungicide on different organism groups and ecosystem functioning in a stream detrital food web. *Aquatic Toxicology*. 2017;186:215–21. <https://doi.org/10.1016/j.aquatox.2017.03.008>.
 60. Cui N, Xu H, Yao S, He Y, Zhang H, Yu Y. Chiral triazole fungicide tebuconazole: Enantioselective bioaccumulation, bioactivity, acute toxicity and dissipation in soils. *Environ Sci Pollut Res*. 2018;25(25):25468–75. <https://doi.org/10.1007/s11356-018-2587-9>.
 61. Wang T, Zhang H, Zhu H. CRISPR Technology is revolutionizing the improvement of tomato and other fruit crops. *Hortic Res*. 2019;6(1):77. <https://doi.org/10.1038/s41438-019-0159-x>.
 62. Zhou J, Zhang J, Li F, Liu J. Triazole fungicide tebuconazole disrupts human placental trophoblast cell functions. *J Hazard Mater*. 2016;308:294–302. <https://doi.org/10.1016/j.jhazmat.2016.01.055>.
 63. Pirozzi AVA, Stellavato A, La Gatta A, Lamberti M, Schiraldi C. Mancozeb, a fungicide routinely used in agriculture, worsens nonalcoholic fatty liver disease in the human Hepg2 cell model. *Toxicol Lett*. 2016;249:1–4. <https://doi.org/10.1016/j.toxlet.2016.03.004>.
 64. Paro R, Tiboni GM, Buccione R, Rossi G, Cellini V, Canipari R et al. The fungicide mancozeb induces toxic effects on mammalian granulosa cells. *Toxicol Appl Pharmacol*. 2012;260(2):155–61. <https://doi.org/10.1016/j.taap.2012.02.005>.
 65. Walia A, Mehta P, Guleria S, Chauhan A, Shirkot CK. Impact of fungicide mancozeb at different application rates on soil microbial populations, soil biological processes and enzyme activities in soil. *Sci World J*. 2014;2014:1–9. <https://doi.org/10.1155/2014/702909>.
 66. Andersen E, Ali S, Byamukama E, Yen Y, Nepal M. Disease resistance mechanisms in plants. *Genes (Basel)*. 2018;9(7):339. <https://doi.org/10.3390/genes9070339>.
 67. Kamle M, Kumar P, Patra JK, Bajpai VK. Current perspectives on genetically modified crops and detection methods. *3 Biotech*. 2017;7(3):219. <https://doi.org/10.1007/s13205-017-0809-3>.
 68. Araus JL, Serret MD, Lopes MS. Transgenic solutions to increase yield and stability in wheat: Shining hope or flash in the pan? *J Exp Bot*. 2019;70(5):1419–24. <https://doi.org/10.1093/jxb/erz077>.
 69. Das P, Adak S, Lahiri Majumder A. Genetic manipulation for improved nutritional quality in rice. *Front Genet*. 2020;11. <https://doi.org/10.3389/fgene.2020.00776>.
 70. Yang, Kloos, Mora-Ramírez, Romeis, Brunner, Li et al. Transgenic winter wheat expressing the sucrose transporter HvSUT1 from barley does not affect aphid performance. *Insects*. 2019;10(11):388. <https://doi.org/10.3390/insects10110388>.
 71. Yang X, Yang J, Wang Y, He H, Niu L, Guo D et al. Enhanced resistance to *Sclerotinia* stem rot in transgenic soybean that overexpresses a wheat oxalate oxidase. *Transgenic Res*. 2019;28(1):103–14. <https://doi.org/10.1007/s11248-018-0106-x>.
 72. Hao G, Bakker MG, Kim H-S. Enhanced resistance to *Fusarium graminearum* in transgenic *Arabidopsis* plants expressing a modified plant Thionin. *Phytopathology®*. 2020; 110(5):1056–66. <https://doi.org/10.1094/PHYTO-12-19-0447-R>.
 73. Wang J, Gao X, Dong J, Tian X, Wang J, Palta JA et al. Overexpression of the heat-responsive wheat gene TaHSP23.9 in transgenic *Arabidopsis* conferred tolerance to heat and salt stress. *Front Plant Sci*. 2020;11. <https://doi.org/10.3389/fpls.2020.00243>.
 74. Dai X, Wang Y, Chen Y, Li H, Xu S, Yang T et al. Overexpression of NtDOG1L-T improves heat stress tolerance by modulation of antioxidant capability and defense, heat and ABA-related gene expression in tobacco. *Front Plant Sci*. 2020 Oct 30;11:568489. <https://doi.org/10.3389/fpls.2020.568489>.
 75. Song X, Li E, Song H, Du G, Li S, Zhu H et al. Genome-wide identification and characterization of nonspecific lipid transfer protein (nsLTP) genes in *Arachis duranensis*. *Genomics*. 2020;112(6):4332–41. <https://doi.org/10.3389/fpls.2020.568489>.
 76. Guo X, Li J, Zhang L, Zhang Z, He P, Wang W et al. Heterotrimeric G-protein α subunit (LeGPA1) confers cold stress tolerance to processing tomato plants (*Lycopersicon esculentum* Mill). *BMC Plant Biol*. 2020;20(1):394. <https://doi.org/10.1186/s12870-020-02615-w>.
 77. Zhou Y, Chen M, Guo J, Wang Y, Min D, Jiang Q et al. Overexpression of soybean DREB1 enhances drought stress tolerance of transgenic wheat in the field. *J Exp Bot*. 2020; 71(6):1842–57. <https://doi.org/10.1093/jxb/erz569>.
 78. Zhao T, Wu T, Pei T, Wang Z, Yang H, Jiang J et al. Overexpression of SIGATA17 promotes drought tolerance in transgenic tomato plants by enhancing activation of the phenylpropanoid biosynthetic pathway. *Front Plant Sci*. 2021;12. <https://doi.org/10.3389/fpls.2021.634888>.
 79. Chenault, K.D., Melouk, H.A., Payton and ME. Effect of anti-fungal transgene (S) on agronomic traits of transgenic peanut lines grown under field conditions. *Peanut Sci*. 2006;33(1):12–19. [https://doi.org/10.3146/0095-3679\(2006\)33\[12:EOATOA\]2.0.CO;2](https://doi.org/10.3146/0095-3679(2006)33[12:EOATOA]2.0.CO;2).
 80. Jackson SA, Iwata A, Lee S, Schmutz J, Shoemaker R. Sequencing crop genomes: Approaches and applications. *New Phytol*. 2011;191(4):915–25. <https://doi.org/10.1111/j.1469-8137.2011.03804.x>.
 81. Chenault KD, Burns JA, Melouk HA, Payton ME. Hydrolase activity in transgenic peanut. *Peanut Sci*. 2002;29(2):89–95. <https://doi.org/10.3146/pnut.29.2.0003>.
 82. Krishna G, Singh BK, Kim E-K, Morya VK, Ramteke PW. Progress in genetic engineering of peanut (*Arachis hypogaea* L.)-A review. *Plant Biotechnol J*. 2015;13(2):147–62. <https://doi.org/10.1111/pbi.12339>.
 83. Ozias-Akins P, Schnall JA, Anderson WF, Singsit C, Clemente TE, Adang MJ et al. Regeneration of transgenic peanut plants from stably transformed embryogenic callus. *Plant Sci*. 1993;93(1–2):185–94. [https://doi.org/10.1016/0168-9452\(93\)90048-5](https://doi.org/10.1016/0168-9452(93)90048-5).

84. Mallikarjuna G, Rao TSRB, Kirti PB. Genetic engineering for peanut improvement: Current status and prospects. *Plant Cell, Tissue Organ Cult.* 2016;125(3):399–416. <https://doi.org/10.1007/s11240-016-0966-9>.
85. Pickova D, Ostry V, Malir F. A recent overview of producers and important dietary sources of aflatoxins. *Toxins (Basel)*. 2021 Mar 3;13(3):186. Available from: <https://www.mdpi.com/2072-6651/13/3/186>.
86. Kimanya ME, Routledge MN, Mpolya E, Ezekiel CN, Shirima CP, Gong YY. Estimating the risk of aflatoxin-induced liver cancer in Tanzania based on biomarker data. *PLoS One*. 2021;16(3):e0247281:1-11. <https://doi.org/10.3390/toxins13030186>.
87. Haque E, Peter AV, Jairath MS. Tackling aflatoxin in groundnut. *The Hindu, Business Line*. [Internet] 2018 Jan 24 [Cited 2021 Sep 1]. Available from: <https://www.thehindubusinessline.com/markets/commodities/tackling-aflatoxin-in-groundnut/article6861407.ece>.
88. Dawlatana M, Coker RD, Nagler MJ, Wild CP, Hassan MS, Blunden G. The Occurrence of mycotoxins in key commodities in Bangladesh: Surveillance Results From 1993 to 1995. *J Nat Toxins*. 2002;11(4):379–86.
89. Ozias-Akins P, Yang H, Roberson E, Akasaka E, Lynch R. Genetic engineering of peanut for reduction of aflatoxin contamination [Internet]. United States Department of Agriculture. Research, Education and Economic Information System; 2000 [Cited 2021 Sep 1]. Available from: <https://portal.nifa.usda.gov/web/crisprojectpages/0410690-genetic-engineering-of-peanut-for-reduction-of-aflatoxin-contamination.html>.
90. Niu C, Akasaka-Kennedy Y, Faustinielli P, Joshi M, Rajasekaran K, Yang H et al. Antifungal activity in transgenic peanut (*Arachis hypogaea* L.) conferred by a nonheme chloroperoxidase gene. *Peanut Sci.* 2009;36(2):126–32. <https://doi.org/10.3146/PS08-020.1>.
91. Sundaresha S, Manoj Kumar A, Rohini S, Math SA, Keshamma E, Chandrashekar SC et al. Enhanced protection against two major fungal pathogens of groundnut, *Cercospora arachidicola* and *Aspergillus flavus* in transgenic groundnut over-expressing a tobacco β 1–3 glucanase. *Eur J Plant Pathol*. 2010;126(4):497–508. <https://doi.org/10.1007/s10658-009-9556-6>.
92. Sharma KK, Pothana A, Prasad K, Shah D, Kaur J, Bhatnagar D et al. Peanuts that keep aflatoxin at bay: a threshold that matters. *Plant Biotechnol J.* 2018;16(5):1024–33. <https://doi.org/10.1111/pbi.12846>.
93. Arias RS, Dang PM, Sobolev VS. RNAi-mediated control of aflatoxins in peanut: Method to analyze mycotoxin production and transgene expression in the peanut/*Aspergillus* pathosystem. *J Vis Exp*. 2015;106:1-11. <https://doi.org/10.3791/53398>.
94. Jayaprakash A, Thanmalagan RR, Roy A, Arunachalam A, Lakshmi P. Strategies to understand *Aspergillus flavus* resistance mechanism in *Arachis hypogaea* L. *Current Plant Biol.* 2019;20:100123:1-11. <https://doi.org/10.1016/j.cpb.2019.100123>.
95. Stahl EA, Bishop JG. Plant-pathogen arms races at the molecular level. *Curr Opin Plant Biol.* 2000;3(4):299–304. [https://doi.org/10.1016/S1369-5266\(00\)00083-2](https://doi.org/10.1016/S1369-5266(00)00083-2).
96. Chenault KD, Payton ME, Melouk HA. Greenhouse testing of transgenic peanut for resistance to *Sclerotinia minor*. *Peanut Sci.* 2003;30(2):116–20. <https://doi.org/10.3146/pnut.30.2.0011>.
97. Punja ZK, Zhang YY. Plant chitinases and their roles in resistance to fungal diseases. *J Nematol.* 1993;25(4):526–40.
98. Prasad K, Bhatnagar-Mathur P, Waliyar F, Sharma KK. Overexpression of a chitinase gene in transgenic peanut confers enhanced resistance to major soil borne and foliar fungal pathogens. *J Plant Biochem Biotechnol.* 2013;22(2):222–33. <https://doi.org/10.1007/s13562-012-0155-9>.
99. Rohini V, Sankara Rao K. Transformation of peanut (*Arachis hypogaea* L.) with tobacco chitinase gene: Variable response of transformants to leaf spot disease. *Plant Science.* 2001;160(5):889–98. [https://doi.org/10.1016/S0168-9452\(00\)00462-3](https://doi.org/10.1016/S0168-9452(00)00462-3).
100. Iqbal MM, Yusuf Z, Farhat N, Shaukat A, Javaid I, Asif MA, Omer R, Ali GM. Over expression of bacterial chitinase gene in Pakistani peanut (*Arachis hypogaea* L.) cultivar GOLDEN. *Afr J Biotechnol.* 2011;10(31):5838–44. <https://doi.org/10.5897/AJB11.681>.
101. Iqbal MM, Nazir F, Ali S, Asif MA, Zafar Y, Iqbal J et al. Over expression of rice chitinase gene in transgenic peanut (*Arachis hypogaea* L.) improves resistance against leaf spot. *Mol Biotechnol.* 2012;50(2):129–36. <https://doi.org/10.1007/s12033-011-9426-2>.
102. Grison R, Grezes-Besset B, Schneider M, Lucante N, Olsen L, Leguay JJ et al. Field tolerance to fungal pathogens of *Brassica napus* constitutively expressing a chimeric chitinase gene. *Nat Biotechnol.* 1996;14(5):643–46. <https://doi.org/10.1038/nbt0596-643>.
103. Van Leeuwen W, Ruttink T, Borst-Vrens AWM, van der Plas LHW, van der Krol AR. Characterization of position-induced spatial and temporal regulation of transgene promoter activity in plants. *J Exp Bot.* 2001;52(358):949–59. <https://doi.org/10.1093/jexbot/52.358.949>.
104. Qiao LX, Ding X, Wang HC, Sui JM, Wang J-S. Characterization of the β -1,3-glucanase gene in peanut (*Arachis hypogaea* L.) by cloning and genetic transformation. *Genet Mol Res.* 2014;13(1):1893–904. <https://doi.org/10.4238/2014.March.17.17>
105. Livingstone DM, Hampton JL, Phipps PM, Grabau EA. Enhancing resistance to *Sclerotinia minor* in peanut by expressing a barley oxalate oxidase gene. *Plant Physiol.* 2005;137(4):1354–62. <https://doi.org/10.1104/pp.104.057232>.
106. Sinha M, Singh RP, Kushwaha GS, Iqbal N, Singh A, Kaushik S et al. Current overview of allergens of plant pathogenesis related protein families. *Sci World J.* 2014;2014:1–19. <https://doi.org/10.1155/2014/543195>.
107. Pusztahelyi T. Chitin and chitin-related compounds in plant-fungal interactions. *Mycology.* 2018;9(3):189–201. <https://doi.org/10.1080/21501203.2018.1473299>.
108. Swathi Anuradha T, Divya K, Jami SK, Kirti PB. Transgenic tobacco and peanut plants expressing a mustard defensin show resistance to fungal pathogens. *Plant Cell Rep.* 2008; 27(11):1777–86. <https://doi.org/10.1007/s00299-008-0596-8>.
109. Hasan MM, Islam R, Hossain I, Shirin K. Biological control of leaf spot of groundnut. *J Biosci Agric Res.* 2014;1(2):66–78. <https://doi.org/10.18801/jbar.010214.08>.
110. Vasavirama K, Kirti PB. Increased resistance to late leaf spot disease in transgenic peanut using a combination of PR genes. *Funct Integr Genomics.* 2012;12(4):625–34. <https://doi.org/10.1007/s10142-012-0298-8>.
111. Kumar D, Kirti PB. Transcriptomic and proteomic analyses of resistant host responses in *Arachis diogeni* challenged with late leaf spot pathogen, *Phaeoisariopsis personata*. *PLoS One.* 2015;10(2):e0117559:1-29. <https://doi.org/10.1371/journal.pone.0117559>.
112. Kumar D, Kirti PB. Pathogen-induced SGT1 of *Arachis diogeni* induces cell death and enhanced disease resistance in tobacco and peanut. *Plant Biotechnol J.* 2015;13(1):73–84. <https://doi.org/10.1111/pbi.12237>.
113. Hassan M ul, Akram Z, Ali S, Ali GM, Zafar Y, Shah ZH et al. Whisker-mediated transformation of peanut with chitinase gene enhances resistance to leaf spot disease. *Crop Breed Appl Biotechnol.* 2016;16(2):108–14. <https://doi.org/10.1590/1984-70332016v16n2a17>.
114. Hain R, Bieseler B, Kindl H, Schröder G, Stöcker R. Expression of a stilbene synthase gene in *Nicotiana tabacum* results in synthesis

- of the phytoalexin resveratrol. *Plant Mol Biol.* 1990;15(2):325–35. <https://doi.org/10.1007/BF00036918>.
115. Altpeter F, Springer NM, Bartley LE, Blechl A, Brunnell TP, Citovsky V et al. Advancing crop transformation in the era of genome editing. *Plant Cell.* 2016;28(7):1510–20. <https://doi.org/10.1105/tpc.16.00196>.
 116. Das Bhowmik SS, Cheng AY, Long H, Tan GZH, Hoang TML, Karbaschi MR et al. Robust genetic transformation system to obtain non-chimeric transgenic chickpea. *Front Plant Sci.* 2019;10. <https://doi.org/10.3389/fpls.2019.00524>.
 117. Arencibia AD, D'Afonseca V, Chakravarthi M, Castiglione S. Learning from transgenics: Advanced gene editing technologies should also bridge the gap with traditional genetic selection. *Electron J Biotechnol.* 2019;41:22–29. <https://doi.org/10.1016/j.ejbt.2019.06.001>.
 118. Prasad K, Bhatnagar-Mathur P, Narasu ML, Waliyar F, Sharma KK. Transgenic approaches for improving fungal disease resistance in groundnut. *Technol Spectr.* 2011;5(1):1–10.
 119. McCabe DE, Swain WF, Martinell BJ, Christou P. Stable transformation of soybean (*Glycine max*) by particle acceleration. *Nat Biotechnol.* 1988; 6(8):923–26. <https://doi.org/10.1038/nbt0888-923>.
 120. Somers DA, Samac DA, Olhoft PM. Recent advances in legume transformation. *Plant Physiol.* 2003;131(3):892–99. <https://doi.org/10.1104/pp.102.017681>.
 121. Teixeira da Silva JA, Nezami-Alanagh E, Barreal ME, Kher MM, Wicaksono A, Gulyás A et al. Shoot tip necrosis of *in vitro* plant cultures: a reappraisal of possible causes and solutions. *Planta.* 2020;252(3):47. <https://doi.org/10.1007/s00425-020-03449-4>.
 122. Perl A, Lotan O, Abu-Abied M, Holland D. Establishment of an agrobacterium-mediated transformation system for grape (*Vitis vinifera* L.): The role of antioxidants during grape–agrobacterium interactions. *Nat Biotechnol.* 1996;14(5):624–28. <https://doi.org/10.1038/nbt0596-624>.
 123. Enriquez-Obregon GA, Vazquez-Padron RI, Prieto-Samsonov DL, Perez M, Selman-Housein G. Genetic transformation of sugarcane by *Agrobacterium tumefaciens* using antioxidant compounds. *Biotechnol Appl.* 1997;14(3):169–74.
 124. Enríquez-Obregón GA, Prieto-Samsónov DL, Gustavo A, Pérez M, Selman-Housein G, Vázquez-Padrón RI. Agrobacterium-mediated japonica rice transformation: a procedure assisted by an antinecrotic treatment. *Plant Cell Tissue Organ Cult.* 1999;59:159–69. <https://doi.org/10.1023/A:1006307527449>.
 125. Sarmah BK, Moore A, Tate W, Molvig L, Morton RL, Rees DP et al. Transgenic chickpea seeds expressing high levels of a bean - amylase inhibitor. *Mol Breed.* 2004;14(1):73–82. <https://doi.org/10.1023/B:MOLB.0000037996.01494.12>
 126. CelikkolAkcaay U, Mahmoudian M, Kamci H, Yucel M, Oktem HA. Agrobacterium tumefaciens-mediated genetic transformation of a recalcitrant grain legume, lentil (*Lens culinaris* Medik). *Plant Cell Rep.* 2009;28(3):407–17. <https://doi.org/10.1007/s00299-008-0652-4>.
 127. Prasad K, Pooja Bhatnagar-mathur, Narasu ML, Farid Waliyar KKS. Transgenic approaches for improving fungal disease resistance in groundnut. *Technol Spectr.* 2011;5(1):1–10.
 128. Schürholz A-K, López-Salmerón V, Li Z, Forner J, Wenzl C, Gailloch C et al. A comprehensive toolkit for inducible, cell type-specific gene expression in *Arabidopsis*. *Plant Physiol.* 2018;178(1):40–53. <https://doi.org/10.1104/pp.18.00463>.
 129. Scharl CL, Byrd AD, Benzion G, Altschuler MA, Hildebrand DF, Hunt AG. Design and construction of a versatile system for the expression of foreign genes in plants. *Gene.* 1987;61(1):1–11. [https://doi.org/10.1016/0378-1119\(87\)90359-3](https://doi.org/10.1016/0378-1119(87)90359-3).
 130. Garbarino JE, Belknap WR. Isolation of a ubiquitin-ribosomal protein gene (ubi3) from potato and expression of its promoter in transgenic plants. *Plant Mol Biol.* 1994;24(1):119–27. <https://doi.org/10.1007/BF00040579>.
 131. Oyelakin OO, Opabode JT, Raji AA, Ingelbrecht IL. A Cassava vein mosaic virus promoter cassette induces high and stable gene expression in clonally propagated transgenic cassava (*Manihot esculenta* Crantz). *S Afr J Bot.* 2015. ;97:184-90.<https://doi.org/10.1016/j.sajb.2014.11.011>.
 132. Sunilkumar G, Connell JP, Smith CW, Reddy AS, Rathore KS. Cotton alpha-globulin promoter: Isolation and functional characterization in transgenic cotton, *Arabidopsis* and tobacco. *Transgenic Res.* 2002;11(4):347–59. <https://doi.org/10.1023/A:1016322428517>.
 133. Skadsen RW, Sathish P, Federico ML, Abebe T, Fu J, Kaeppler HF. Cloning of the promoter for a novel barley gene, Lem1 and its organ-specific promotion of Gfp expression in lemma and palea. *Plant Molecular Biology.* 2002;49(5):545–55. <https://doi.org/10.1023/A:1015509400123>.
 134. Yuan C, Li C, Yan C, Zhao X, Wang J, Sun Q et al. Isolation and characterization of a novel seed-specific promoter from peanut (*Arachis hypogaea* L.). *Mol Biol Rep.* 2019;46(3):3183–91. <https://doi.org/10.1007/s11033-019-04775-x>.
 135. Tang G, Xu P, Ma W, Wang F, Liu Z, Wan S et al. Seed-specific expression of AtLEC1 increased oil content and altered fatty acid composition in seeds of peanut (*Arachis hypogaea* L.). *Front Plant Sci.* 2018;9:260. <https://doi.org/10.3389/fpls.2018.00260>.
 136. Tamayo MC, Rufat M, Bravo JM, San Segundo B. Accumulation of a maize proteinase inhibitor in response to wounding and insect feeding and characterization of its activity toward digestive proteinases of *Spodoptera littoralis* larvae. *Planta.* 2000;211(1):62–71. <https://doi.org/10.1007/s004250000258>.
 137. Yevtushenko DP, Sidorov VA, Romero R, Kay WW, Misra S. Wound-inducible promoter from poplar is responsive to fungal infection in transgenic potato. *Plant Sci.* 2004; 167(4):715–24. <https://doi.org/10.1016/j.plantsci.2004.04.023>.
 138. Low LY, Yang SK, Andrew Kok DX, Ong-Abdullah J, Tan NP, Lai KS. Transgenic plants: Gene constructs, vector and transformation method. In: Çelik Ö, editor. *New Visions in Plant Science*. London: Intech Open; 2018. p. 41-61. <https://doi.org/10.5772/intechopen.79369>.
 139. Gupta D, Dey N, Leelavathi S, Ranjan R. Development of efficient synthetic promoters derived from pararetrovirus suitable for translational research. *Planta.* 2021;253(2):42. <https://doi.org/10.1007/s00425-021-03565-9>.
 140. Punja ZK. Genetic engineering of plants to enhance resistance to fungal pathogens- A review of progress and future prospects. *Can J Plant Pathol.* 2001;23(3):216–35. <https://doi.org/10.1080/07060660109506935>.
 141. Guo M, Ye J, Gao D, Xu N, Yang J. Agrobacterium-mediated horizontal gene transfer: Mechanism, biotechnological application, potential risk and forestalling strategy. *Biotechnol Adv.* 2019;37(1):259–70. <https://doi.org/10.1016/j.biotechadv.2018.12.008>.
 142. Nonaka S, Someya T, Kadota Y, Nakamura K, Ezura H. Super-agrobacterium ver. 4: Improving the transformation frequencies and genetic engineering possibilities for crop plants. *Front Plant Sci.* 2019;10:1204:1-12. <https://doi.org/10.3389/fpls.2019.01204>.
 143. Soni P, Gangurde SS, Ortega-Beltran A, Kumar R, Parmar S, Sudini HK et al. Functional biology and molecular mechanisms of host-pathogen interactions for aflatoxin contamination in groundnut (*Arachis hypogaea* L.) and maize (*Zea mays* L.). *Front Microbiol.* 2020;11:227:1-22. <https://doi.org/10.3389/fmicb.2020.00227>.
 144. Xi J, Patel M, Dong S, Que Q, Qu R. Acetosyringone treatment duration affects large T-DNA molecule transfer to rice callus. *BMC*

- Biotechnol. 2018;18(1):48. <https://doi.org/10.1186/s12896-018-0459-5>.
145. Marka R, Nanna RS. Optimization of factors affecting *Agrobacterium*-mediated genetic transformation in groundnut (*Arachis hypogaea* L.). *Adv Plants Agric Res*. 2018;8(3):275–82. <https://doi.org/10.15406/apar.2018.08.00327>.
 146. Sivanandhan G, Kapil Dev G, Thebora J, Selvaraj N, Ganapathi A, Manickavasagam M. Sonication, vacuum infiltration and thiol compounds enhance the agrobacterium-mediated transformation frequency of *Withania somnifera* (L.) Dunal. *Munderloh UG*, editor. *PLoS One*. 2015;10(4):e0124693:1-23. <https://doi.org/10.1371/journal.pone.0124693>.
 147. Matheka J, Tripathi JN, Merga I, Gebre E, Tripathi L. A simple and rapid protocol for the genetic transformation of *Ensete ventricosum*. *Plant Methods*. 2019;15(1):130. <https://doi.org/10.1186/s13007-019-0512-y>.
 148. Yadava P, Abhishek A, Singh R, Singh I, Kaul T, Pattanayak A et al. Advances in maize transformation technologies and development of transgenic maize. *Front Plant Sci*. 2017;7:1949:1-12 <https://doi.org/10.3389/fpls.2016.01949>.
 149. Khan MS, Mustafa G, Joyia FA. Technical Advances in Chloroplast Biotechnology. In: Khan MS, Malik KA editors. *Transgenic crops - emerging trends and future perspectives*. London: Intech Open; 2019. p.1-13. <https://doi.org/10.5772/intechopen.81240>.
 150. Mustafa G, Khan MS. Transmission of engineered plastids in sugarcane, a C4 monocotyledonous plant, reveals that sorting of preprogrammed progenitor cells produce heteroplasmy. *Plants*. 2020;10(1):26. <https://doi.org/10.3390/plants10010026>.
 151. Peng Z, Liu F, Wang L, Zhou H, Paudel D, Tan L et al. Transcriptome profiles reveal gene regulation of peanut (*Arachis hypogaea* L.) nodulation. *Sci Rep*. 2017;7(1):40066:1-12. <https://doi.org/10.1038/srep40066>.
 152. Agarwal G, Clevenger J, Pandey MK, Wang H, Shasidhar Y, Chu Y et al. High density genetic map using whole genome resequencing for fine mapping and candidate gene discovery for disease resistance in peanut. *Plant Biotechnol J*. 2018;16(11):1954–67. <https://doi.org/10.1111/pbi.12930>.
 153. Gong L, Han S, Yuan M, Ma X, Hagan A, He G. Transcriptomic analyses reveal the expression and regulation of genes associated with resistance to early leaf spot in peanut. *BMC Res Notes*. 2020;13(1):381. <https://doi.org/10.1186/s13104-020-05225-9>.
 154. Payton P, Kottapalli K, Rowland D, Faircloth W, Guo B, Burow M et al. Gene expression profiling in peanut using high density oligonucleotide microarrays. *BMC Genomics*. 2009; 10(1):265. <https://doi.org/10.1186/1471-2164-10-265>.
 155. Feng S, Wang X, Zhang X, Dang PM, Holbrook CC, Culbreath AK et al. Peanut (*Arachis hypogaea*) expressed sequence tag project: Progress and application. *Comp Funct Genomics*. 2012;2012:1–9. <https://doi.org/10.1155/2012/373768>.
 156. Janila P, Variath MT, Pandey MK, Desmae H, Motagi BN, Okori P et al. Genomic tools in groundnut breeding program: Status and perspectives. *Front Plant Sci*. 2016;7:289:1-15. <https://doi.org/10.3389/fpls.2016.00289>.
 157. Lv Y, Zhang X, Luo L, Yang H, Li P, Zhang K et al. Characterization of glycerol-3-phosphate acyltransferase 9 (AhGPAT9) genes, their allelic polymorphism and association with oil content in peanut (*Arachis hypogaea* L.). *Sci Rep*. 2020;10(1):14648:1-15. <https://doi.org/10.1038/s41598-020-71578-7>.
 158. Noblie PL. Identification and characterization of peanut (*Arachis hypogaea* L.) EST's regulated during interaction with *Cercosporidium personatum* (Berk. & Curt.): XIV International Plant and Animal Genome Conference, San Diego, California, USA; 2006 Jan 14-15. p. 680.
 159. Rathod V, Hamid R, Tomar RS, Padhiyar S, Kheni J, Thirumalaisamy P et al. Peanut (*Arachis hypogaea*) transcriptome revealed the molecular interactions of the defense mechanism in response to early leaf spot fungi (*Cercospora arachidicola*). *Plant Gene*. 2020;23:100243:1-12. <https://doi.org/10.1016/j.plgene.2020.100243>.
 160. Bosamia TC, Dodia SM, Mishra GP, Ahmad S, Joshi B, Thirumalaisamy PP et al. Unraveling the mechanisms of resistance to *Sclerotium rolfsii* in peanut (*Arachis hypogaea* L.) using comparative RNA-seq analysis of resistant and susceptible genotypes. Zhang Z, editor. *PLoS One*. 2020;15(8): e0236823:1-20. <https://doi.org/10.1371/journal.pone.0236823>.
 161. Jogi A, Kerry JW, Brenneman TB, Leebens-Mack JH, Gold SE. Identification of genes differentially expressed during early interactions between the stem rot fungus (*Sclerotium rolfsii*) and peanut (*Arachis hypogaea*) cultivars with increasing disease resistance levels. *Microbiol Res*. 2016;184:1–12. <https://doi.org/10.1016/j.micres.2015.11.003>.
 162. Yu B, Huai D, Huang L, Kang Y, Ren X, Chen Y et al. Identification of genomic regions and diagnostic markers for resistance to aflatoxin contamination in peanut (*Arachis hypogaea* L.). *BMC Genet*. 2019;20(1):32. <https://doi.org/10.1186/s12863-019-0734-z>.
 163. Hao K, Wang F, Nong X, McNeill MR, Liu S, Wang G et al. Response of peanut *Arachis hypogaea* roots to the presence of beneficial and pathogenic fungi by transcriptome analysis. *Sci Rep*. 2017;7(1):964. <https://doi.org/10.1038/s41598-017-01029-3>.
 164. Clevenger J, Chu Y, Chavarro C, Botton S, Culbreath A, Isleib TG et al. Mapping late leaf spot resistance in peanut (*Arachis hypogaea*) using QTL-seq reveals markers for marker-assisted selection. *Front Plant Sci*. 2018;9:83:1-10. <https://doi.org/10.3389/fpls.2018.00083>.
 165. Kumar J, Sen Gupta D, Baum M, Varshney RK, Kumar S. Genomics assisted lentil breeding: Current status and future strategies. *Legum Sci*. 2021;3:e71:1-20. <https://doi.org/10.1002/leg3.71>.
 166. Cui R, Clevenger J, Chu Y, Brenneman T, Isleib TG, Holbrook CC et al. Quantitative trait loci sequencing-derived molecular markers for selection of stem rot resistance in peanut. *Crop Sci*. 2020;60(4):2008–18. <https://doi.org/10.1002/csc2.20047>.
 167. Moradi A, El-Shetehy M, Gamir J, Austerlitz T, Dahlin P, Wiczorek K et al. Expression of a fungal lectin in *Arabidopsis* enhances plant growth and resistance toward microbial pathogens and a plant-parasitic nematode. *Front Plant Sci*. 2021;12:657451:1-13. <https://doi.org/10.3389/fpls.2021.657451>.
 168. Chandrasekaran G, Lee YC, Park H, Wu Y, Shin HJ. Antibacterial and antifungal activities of lectin extracted from fruiting bodies of the Korean cauliflower medicinal mushroom, *Sparassis latifolia* (Agaricomycetes). *Int J Med Mushrooms*. 2016;18(4):291–99. <https://doi.org/10.1615/IntJMedMushrooms.v18.i4.20>.
 169. Chen ZY, Brown RL, Guo BZ, Menkir A, Cleveland TE. Identifying aflatoxin resistance-related proteins/genes through proteomics and RNAi gene silencing1. *Peanut Sci*. 2009;36(1):35–41. <https://doi.org/10.3146/AT07-005.1>.
 170. Halder M, Jha S. Enhanced trans-resveratrol production in genetically transformed root cultures of peanut (*Arachis hypogaea* L.). *Plant Cell, Tissue Organ Cult*. 2016;124(3):555–72. <https://doi.org/10.1007/s11240-015-0914-0>.
 171. Power IL, Faustinelli PC, Orner VA, Sobolev VS, Arias RS. Analysis of small RNA populations generated in peanut leaves after exogenous application of dsRNA and dsDNA targeting aflatoxin synthesis genes. *Sci Rep*. 2020;10(1):13820:1-12. <https://doi.org/10.1038/s41598-020-70618-6>.
 172. Werner BT, Gaffar FY, Schuemann J, Biedenkopf D, Koch AM. RNA-spray-mediated silencing of *Fusarium graminearum* AGO and DCL genes improve barley disease resistance. *Front Plant Sci*. 2020;11:476:1-11. <https://doi.org/10.3389/fpls.2020.00476>.
 173. Forster H, Shuai B. Exogenous siRNAs against chitin synthase gene suppress the growth of the pathogenic fungus *Macrophomi-*

- na phaseolina*. *Mycologia*. 2020;112(4):699–710. <https://doi.org/10.1080/00275514.2020.1753467>.
174. Nerva L, Sandrini M, Gambino G, Chitarra W. Double-stranded RNAs (dsRNAs) as a sustainable tool against gray mold (*Botrytis cinerea*) in grapevine: Effectiveness of different application methods in an open-air environment. *Biomolecules*. 2020;10(2):200. <https://doi.org/10.3390/biom10020200>.
175. McLoughlin AG, Wytinck N, Walker PL, Girard IJ, Rashid KY, de Kievit T et al. Identification and application of exogenous dsRNA confers plant protection against *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *Sci Rep*. 2018;8(1):7320:1-14. <https://doi.org/10.1038/s41598-018-25434-4>.
176. Song X-S, Gu K-X, Duan X-X, Xiao X-M, Hou Y-P, Duan Y-B et al. Secondary amplification of siRNA machinery limits the application of spray-induced gene silencing. *Mol Plant Pathol*. 2018;19(12):2543–60. <https://doi.org/10.1111/mpp.12728>.
177. Cooper B, Campbell KB. Protection against common bean rust conferred by a gene-silencing method. *Phytopathology*. 2017;107(8):920–27. <https://doi.org/10.1094/PHYTO-03-17-0095-R>.
178. Jahan SN, Asman AKM, Corcoran P, Fogelqvist J, Vetukuri RR, Dixelius C. Plant-mediated gene silencing restricts growth of the potato late blight pathogen *Phytophthora infestans*. *J Exp Bot*. 2015;66(9):2785–94. <https://doi.org/10.1093/jxb/erv094>.

§§§