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Breeding for foliar disease resistance in groundnut

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Introduction

The cultivated groundnut, *Arachis hypogaea* L. is an allotetraploid species (2n = 2x = 40) belongs to family *Fabaceae*, sub-family *Papilionaceae*. The diploid progenitors, *A. duranensis* and *A. ipaensis*, contributed "AA" and "BB" genomes, respectively, to the cultivated groundnut (Kochert *et al*. 1996). It is also known as peanut, earthnut, monkey nut and manila nut, is an important oil, food, and feed legume crop. Groundnut is grown in more than 100 countries with different agro-climatic conditions on about 26.5 million ha with total production of 43.9 million tons and productivity of 1654 kg/ha in 2014 (FAO, 2017). In India, it is cultivated on about 3.7 million ha with the production and productivity of 6.7 million tonne and 1810 kg/ha respectively in 2015-16 (Anonymous, 2017). In India it is grown mainly in Gujarat, Andhra Pradesh, Karnatka, Rajasthan, Tamil Nadu, Maharashtra and Madhya Pradesh. There are two major subspecies of groundnut that also differ in their branching patterns *viz*., *hypogaea* and *fastigiata*. The low yields in peanut are primarily due to low inputs, rainfed cultivation of the crop in marginal lands, non-availability of seed of high-yielding cultivars and the occurrence of insect pests and diseases at different growth stages of the crop (Nigam *et al*. 2012).

Among foliar fungal diseases, early and late leaf spot, rust are economically important diseases in rainy season in India. The productivity of groundnut is high in *rabi* than in *kharif* season crop because of assured irrigation and occurrence of low incidence of biotic stresses. Many biotic stresses are known to limit groundnut productivity during *rabi* season but their severity and distribution vary with prevailing environmental conditions in the region. *Alternaria* leaf blight is a minor foliar fungal disease reported in Gujarat and in southern states viz., Karnataka, Andhra Pradesh and Tamil Nadu during rabi/summer crop. *Alternaria* fungi tend to be weak opportunistic pathogens, whenever crop facing stress such as poor soil fertility, moisture deficit stress, insect damage, and nutrient deficiency also increases disease severity. These diseases can be controlled by fungicides but it is not economic viable approach for poor farmers, it adds input cost of commercial cultivation, is not environment friendly. Therefore develop and breed resistant groundnut varieties for foliar diseases are best and viable approach to minimize economic losses of farmer and maintains good quality of the product.

Economic importance of foliar diseases

Among foliar fungal diseases, three major foliar diseases namely early leaf spot (*Cercospora arachidicola* Hori), late leaf spot [*Phaeoisariopsis personata* (Berk. & Curt.)Van.Arx.] and rust (*Puccinia arachidis* Speg.) are the most widely distributed and economically important diseases of groundnut. Foliar fungal diseases are the major production constraints of groundnut worldwide wherever the crop is grown. These diseases can cause more than 70% loss in yield besides adversely affecting the quality of the produce (pods, seeds and haulms) (Aruna *et al*. 2005). Late leaf spot is a major and widely distributed disease. It can cause

defoliation and reduce pod and fodder yields about 50% and adversely affect quality of its produce (Subrahmanyam *et al*. 1984). Rust is also economic important disease causing yield losses range from 10 - 52%, in addition to a decline in seed quality (Subrahmanyam *et al*. 1995). *Alternaria* leaf spot is also a foliar fungal disease increasing in southern states of countries and in Gujarat during rabi/summer crop. The intensity of this disease varies from 15-35% which appears 30-35 days after sowing Ghewande *et al*. (1982). It has been reported that *Alternaria* leaf blight reduced 13-22% pod yield, 24-63% haulm yield and it also affects kernel quality in groundnut (Kumar et al. 2012).

Early leaf spot: Sub-circular dark brown spots with yellow hello are produced on the upper leaflet surface.

Late leaf spot: Dark brown to black spots are found on the lower leaf surface

Rust: An orange colored pustules that appear on the lower leaflet surface

***Alternaria* leaf blight:** Blighting of apical portion of leaflet, which turn light to dark brown

Genetic improvement for foliar disease resistance

The breeding on foliar diseases was started in early 1970s. Many workers reported sources of resistance to leaf spots in cultivated groundnut. But the level of resistance was not high enough to use them extensively in their breeding programs. Though, high level of resistance or immunity was located in 1974 in wild *Arachis* species for early leaf spot in *A. chacoense* and to late leaf spot in *A. cardenasii* (Abdou *et al*. 1974). First phase of foliar disease resistance breeding programme utilized these resistant sources, PI 259747, PI 298115, EC

76446(292) and NCAc 17133 (RF), resultant breeding lines had high pod and haulm yields but associated with undesirable pod and seed characteristics, low shelling outturn and long duration. Although resistance to rust in these lines was high, the resistance to late leaf spot was diluted from moderate levels to low levels. By utilizing resistant sources, these first generation foliar diseases resistant varieties *viz*., Girnar 1 (1988), ICGS(FDRS) 4 and ICGS (FDRS) 10 (1990) were released in India and elsewhere. However, in spite of high pod and haulm yields under high disease incidence, these varieties not become popular among the farmers due to poor pod and seed characteristics and late maturity. In the second phase of breeding programme, advanced resistant breeding lines were used to develop new resistant cultivars with desirable agronomic characters. This resultant released of three resistant cultivars viz., ICGV 86590 (1991), ICGV 86699, ALR 2 (1994) in India. These cultivars were high yielding with agronomic traits in addition to resistance to rust and tolerance to late leaf spot but crop duration and level of resistance to late leaf spot still remains an unresolved issue (Aruna *et al*. 2005). Efforts were continue to incorporate desirable agronomic traits with reduced crop duration. An inter-specific derivative GPBD-4 (KRG 1 x ICGV 86855) released at U.A.S. Dharwad in 2004, which combined early maturity, high yield potential and high shelling outturn with minimum yield reduction due to high level of resistance to rust and late leaf spot, pod growth rate, partitioning coefficient and harvest index (Gowda*et al*. 2002). Progress in ELS and LLS resistance breeding has been limited by the absence of high levels of resistance in cultivated groundnut and the linkage of resistance with long duration, lower partitioning and with undesirable pod (highly reticulated, constricted, prominently ridged and conspicuously beaked pods with thick shells) and seed (purple or blotched seed color) characteristics (Wynne *et al*.1991, Singh *et al*. 1997). Currently, the emphasis of breeding program is to improve the level of resistance to late leaf spot while maintaining the level of rust resistance in agronomically superior breeding lines in the desired maturity group.

Screening technique for foliar diseases

Effective field and laboratory screening techniques for resistance to leaf spot and rust have been developed by Subrahmanyam *et al*. (1995). Following point should be keep in mind for proper disease development and screening.

1. Inoculum load: Rust and late leaf spot inoculum may be required for areas where the disease pressure is not adequate for a meaningful evaluation of groundnut genotypes for their reactions to these diseases. Field inoculation is also a useful way to achieve uniform disease pressure across the field. Collect spores of rust or late leaf spot pathogens from severely infected groundnut crops plant and multiplied on susceptible cultivars by spraying inoculum.
2. Infector row technique: Infector rows of a highly susceptible cultivar should be grown in the screening trial. Generally a ratio of one infector row to every four rows of test genotypes is adequate. Inoculate infector rows 15 days after sowing and repeat spraying of inoculum depending upon severity of disease sufficient for screening.
3. Scoring: All leaves on the main stem should be examined 2-3 times (75, 90, 105 DAS) up to just before harvest. The modified 9-point scale for leaf spot and rust can also be used for rapid quantification of disease levels (Table and figure 1 & 2).

Table 1: Modified 9-point scale used for field screening groundnut genotypes for resistance to rust

Disease score	Description	Disease severity(%)*
1	No disease	0
2	Pustules sparsely distributed, largely on lower leaves	1-5
3	Many pustules on lower leaves, necrosis evident, very few pustules on middle leaves	6-10
4	Numerous pustules on lower and middle leaves, severe necrosis oflower leaves	11-20
5	Severe necrosis of lower and middle leaves, pustules may be presenton top leaves but less severe	21-30
6	Extensive damage to lower leaves, middle leaves necrotic,withdense distribution of pustules, pustules on top leaves	31-41
7	Severe damage of lower and middle leaves, pustules densely distributed on top leaves	41-60
8	100% damage to lower and middle leaves, pustules on top Leaves, which are severely necrotic	61-80
9	Almost all leaves withered; bare stems seen	81-100
* Percentage leaf area damaged by the disease		

Table 2: Modified 9-point scale used for field screening groundnut genotypes for resistance to late leaf spot

Disease score	Description	Disease severity (%)*
1	No disease	0
2	Lesions present largely on lower leaves, no defoliation	1-5
3	Lesions present largely on lower leaves, very few on middle leaves;defoliation of some leaflets evident on lower leaves	6-10
4	Lesions on lower and middle leaves but severe on lowerleaves, defoliation of some leaf lets evident on lower leaves	11-20
5	Lesions present on all lower and middle leaves, over 50 % defoliationof lower leaves	21-30
6	Severe lesions on lower and middle leaves; lesions present but lesssevere on top leaves; extensive defoliation of lower leaves; defoliation of some leaflet evident on middle leaves	31-41
7	Lesions on all leaves but less severe on top leaves; defoliation of alllower and some middle leaves	41-60
8	Defoliation of all lower and middle leaves; severe lesions on top leaves; somedefoliation of top leaves evident	61-80
9	Almost all leaves defoliated, leaving bare stem; some leaflets mayremain, but show severe leaf spot	81-100
* Percentage leaf area damaged by the disease		

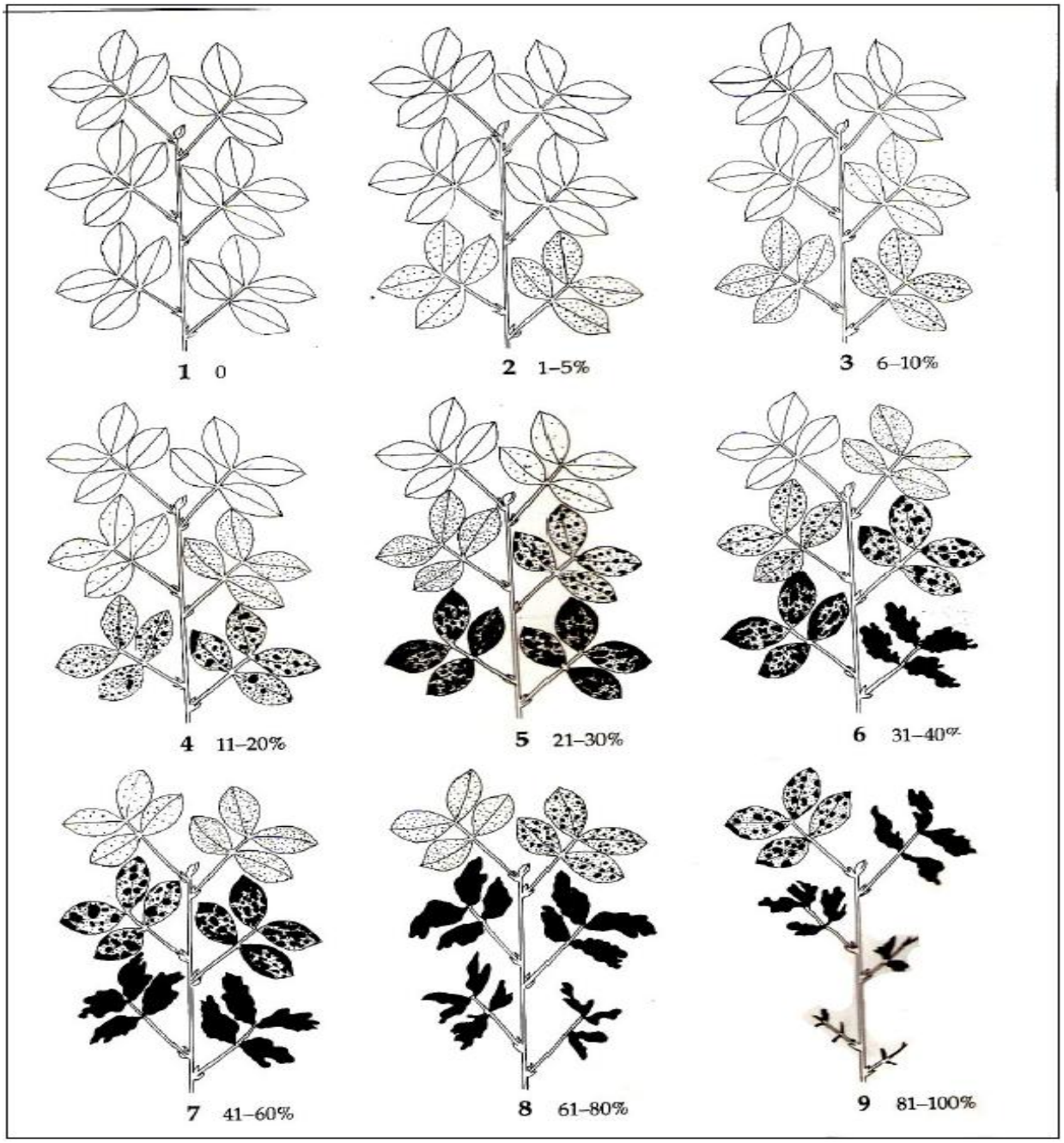

Fig: 1: Modified 9-point scale for field evaluation of rust of groundnut

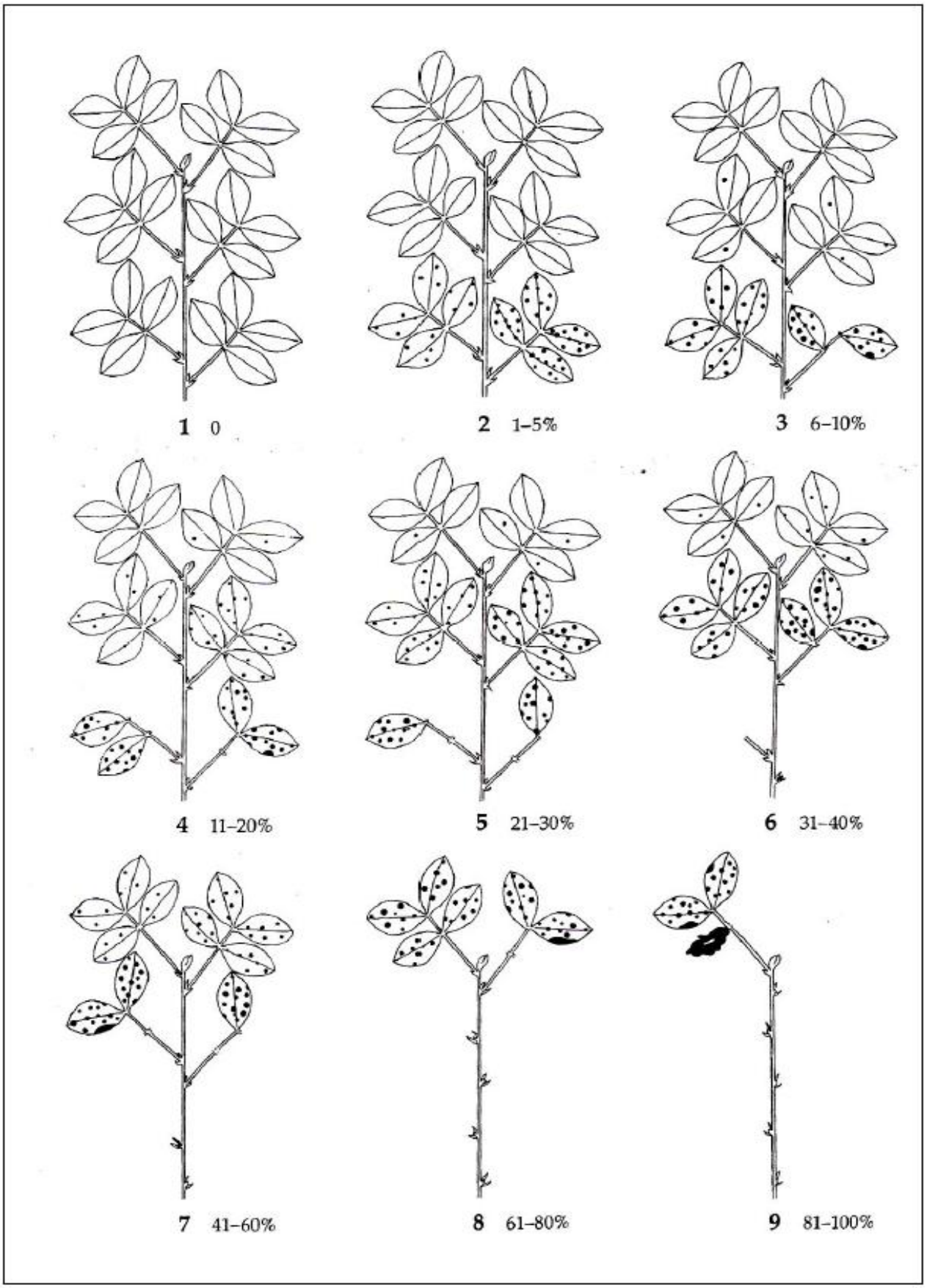

Fig 2: Modified 9-point scale for field evaluation of late leaf spot of groundnut

Genetics of resistance

Most of the ELS resistance sources show significant differences among the components of their resistance. Resistance to ELS is quantitative and controlled predominantly by additive gene effects (Kornegay et al. 1980, Anderson et al. 1986). Narrow-sense heritability estimates have been reported to vary from low to high. Chemical induced physiological races observed which may give differential reaction to resistant sources. Additive and non-additive gene effects and additive x additive gene interaction for resistance with involvement of cytoplasmic factors; Duplicate recessive in induced mutants.

LLS and ELS inherited independently. Resistance to LLS is partial (not complete, as several morphological and anatomical characters of the host plant influence the resistance) and is similar to the 'slow rusting' type of resistance. It operates by prolonging incubation and latent periods, and by reducing the number of lesions per unit area of leaf surface, defoliation, and sporulation. Caldwell (1968), slow rusting is a type of resistance where disease progresses at a retarded rate, resulting in intermediate to low disease levels against all pathotypes of a pathogen. Resistance to leaf spots is recessive and independently inherited. Kornegay et al. (1980) proposed that resistance to leaf spots was quantitatively inherited. Both simple (Tiwari et al. 1984) and complex (Nevill 1982) inheritance of resistance to LLS are reported in the literature. While Tiwari et al. (1984) reported a two-gene control of resistance, Nevill (1982) proposed a five-loci genetic model to explain the inheritance of resistance with the completely recessive alleles determining resistance. Combining ability analysis for components of resistance to LLS indicated the predominant role of additive gene effects for most of the components. Genetic variability for the various components of LLS resistance exists in resistance sources.

The lifecycle of the peanut rust pathogen is incomplete; it is not known whether alternate hosts exist. Instead, the pathogen is highly host specific as there are no reports on hosts outside of the *Arachis* genus. There are no reports on the presence of basidiospores, pycniospores or aeciospores, and teliospores have been rarely observed (Bromfield 1971; Subrahmanyam 1997). Sexual stage and races in groundnut rust pathogen not yet observed. The asexually produced dikaryotic urediniospores are predominant. Little is known about the diversity of the *P. arachidis* fungus, and to our knowledge, little research is being conducted on this subject. Therefore, knowledge on the molecular variability of the pathogen will lay the groundwork in the population structure and evolution of the pathogen. Greater knowledge on the variability of the *P. arachidis* populations and the genetics of resistance to peanut rust will moreover enable us to effectively breed for resistance and thus effectively manage the peanut rust disease on the long run.

There is no complete resistance to *P. arachidis* reported in cultivated peanut. Peanut rust resistance is partial and rate reducing, where several polygenic minor genes, the components of resistance, provide varying levels of partial resistance, leading to a reduced rate of the disease epidemic. The components of peanut rust resistance described are incubation period, latent period, infection frequency, pustule size, percent diseased area, spore production, and spore germination (Bromfield 1971; Cook 1980; Subrahmanyam *et al* 1983).

Resistance to rust in cultivated groundnut is recessive and appears to be governed by only a few genes. One-gene (Paramasivam et al. 1990) and two-gene models (Bromfield and Bailey 1972, Tiwari et al. 1984) have been proposed, but are unable to explain the segregation pattern for rust resistance in many crosses. In interspecific derivatives, rust resistance is governed by partially, dominant gene (s) (Singh et al. 1984). In quantitative genetic analysis, both additive and non-additive gene effects are reported important (Tiwari et al. 1984, Paramasivam et al. 1990). The resistance is stable overs years and locations.

Resistance to rust and LLS is reported to be correlated (r = 0.48 - 0.60) (Anderson et al. 1990). Resistance to rust and LLS is also showed a strong positive association with each other (rp=0.84 and rpg=0.73) which indicating that resistance to both the disease can be incorporated by single breeding effort (Chaudhari *et al*. 2017).

Sources of resistant

Several sources of resistance to LLS have been identified in groundnut (Subrahmanyam et al. 1982; Walls et al. 1985; Anderson et al. 1993; Waliyar et al. 1993; Singh et al. 1997). These genotypes include both wild and cultivated *Arachis* species and their interspecific derivatives. Like rust and LLS resistance sources, most of the ELS resistance sources originated from secondary centers of diversity in South America. But, they have a broader genetic base as several sources of resistance belong to var *hypogaea*, var *fastigiata*, and var *peruviana*. However, none of the sources of resistance is of the Spanish type (var *vulgaris*). Most of these sources show differential disease reactions at different locations, indicating the possible existence of variation in the ELS pathogen. Sources of ELS resistance reported from the USA, were found susceptible when tested at IAC in India and Chitedze in Malawi (Nigam and Bock 1985). Environmental factors, particularly temperature, also affect the stability of the components of resistance to ELS. Several genotypes [91 PA 150, NC Ac 17894 (ICG 6902), PI 274194 (ICG 11476), NC Ac 18045 (ICG 8298), and 91 PA 131], have expressed stable resistance across several temperature regimes (Waliyar et al. 1994).

Above resistant cultivars/genotypes having inferior agronomic traits such as undesirable pod and seed characters, long duration and low shelling out-turn. Because of these characters, they are not become popular among the farmers in spite of their higher yield under disease pressure. Therefore, identification of newer sources of resistance in Spanish types is of great importance in resistance breeding. The aim of breeding programme should be to develop high yielding varieties while maintaining good level of resistance to ELS, LLS and rust. Following improved cultivars/genotypes can be used in foliar disease resistance breeding programme in groundnut.

Table 3: Foliar diseases resistant groundnut genotypes

SN	Disease	Resistant cultivars
1.	Early leaf spot	CSMG-2003-19, GJG-22, GJG-HPS-1, ALR-1, CSMG-884, DSG-1, HNG 123, VRI-5, M-III, JL-776, ALR-3, HNG-10, ICGV 86590, Karad 4-11, T-28, Chitraa, Kaushal, RSB-87, M-197, ICGS-76, RS-138
2.	Late leaf spot	CSMG-884, DSG-1, Faizpur 1-5, MA-16, RG-510, RS-138, T-64

3.	Rust	Girnar-1, FDRS-4, FDRS-10, ICGV-86590, R-2001-2, R-2001-3, ALR-3, VRI-4
4.	LLS and Rust	Dh-4-3, GPBD-4, AK-265, ALR-1, JCG-88, VRI-5, M-III, RHRG-06083, JL-776, KDG-123, KDG-128
5.	*Alternaria* leaf blight	NRCGCS-74, NRCGCS-186, NRCGCS-349

Major constraints to genetic improvement of foliar disease resistance

- ❖ Absence of high levels of resistance in cultivated peanut and the linkage of resistance with long duration, lower partitioning with undesirable pod and seed characteristics
- ❖ Wild *Arachis* spp. showed very high level of resistance to ELS and LLS but possess very small and catenate pods.
- ❖ Limited gene introgression from wild *Arachis* spp. to cultivated groundnut.
- ❖ Disease resistant germplasm are late maturing types, have lower partitioning, and are sensitive to photoperiod than agronomically elite susceptible materials.
- ❖ Large genotype-by-environment interactions for traits of economic importance.

Future prospect of genetic improvement of foliar disease resistance:

- LLS resistance is polygenic in nature, recombination breeding coupled with some amount of recurrent selection to accumulate minor genes in elite susceptible/ tolerant backgrounds may be rewarding.
- Wild species of *Arachis* are known for resistance to LLS and therefore, it would be necessary to include other species as donors to broaden the genetic base of resistance to late leaf spot.
- It is important to use the resistance donor as female parent to tap cytoplasmic inheritance of resistance to LLS.
- Resistance to LLS can be selected from a population of lines which have been selected for resistance to ELS.
- Needed to understand the association between genes (Nuclear or cytoplasmic) responsible for resistance to LLS, ELS and rust
- The preponderance of additive genetic effects for LLS also supports selection for resistance in early generations so possibility of combining high levels of resistance with superior yield and quality factors
- Efforts to overcome incompatibility in wide crosses, by using non-conventional techniques *ie*., Marker-assisted backcross breeding
- DNA marker based genetic linkage map enables breeders to effectively pyramid genes for ELS, LLS and rust into agronomically good cultivars.
- Recombinant inbred lines (RILs) mapping populations should be developed to map the genes for resistance.

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