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BREEDING FOR RESISTANCE TO ASCOCHYTA BLIGHT IN CHICKPEA: SOURCES AND INHERITANCE OF RESISTANCE

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Abstract: In the Mediterranean basin, yield of chickpea is increased by changing the date of sowing of chickpea from spring to winter, resulted in an ascochyta blight [*Ascochyta rabiei* (Pass) Labr.] epidemic. In this review, we addressed to breeder for ascochyta blight and summarized to its importance. Also, importance of sources of resistance, its inheritance and breeding methods for ascochyta blight were emphasized in a breeding programe.

Nohutta Antraknoza Dayanıklılık için Islah: Dayanılılık Kaynakları ve Dayanıklılığın Kalıtımı

Özet: Akdeniz havzasında, nohut verimi ekim tarihinin yazlık ekimden kışlık ekime değişmesiyle artmaktadır. Fakat kışlık ekimlerde antraknoz [*Ascochyta rabiei* (Pass) Labr.] hastalığı ile sonuçlanmaktadır. Biz bu derlemede, ıslahçılara antraknoz hastalığı için yol gösterdik ve hastalığın önemini özetledik. Ayrıca, antraknoz hastalığı için dayanıklılık kaynakları, dayanıklılığın kalıtımı ve ıslah yöntemlerinin önemi bir ıslah programı için vurgulanmıştır.

Introduction

Yield losses in cool season food legumes are resulted from several biotic and abiotic stresses. Those most important of these stresses on chickpea were listed by Nene and Reed (1). Chickpea (*Cicer arietinum* L.) is traditionally grown during the spring season in the Mediterranean region including West Asia, Nort Africa (WANA), and South Europe. In the Mediterranean basin, it is normally sown in spring from late February to early May and grown on soils with residual moisture. The seed yield is restricted by limited moisture availability and spring sowing which coincides with increasing and limiting temparetures during the reproductive phase of the grown (2). Recently, it has been shown that planting in the early winter in the Mediterranean region substantially increases seed yield (2,3,4,5,6). However, winter-sown chickpea must posses resistance to Ascochyta blight [*Ascochyta rabiei* (Pass) Labr.] (2,7,8). Ascochyta blight is the most important foliar disease of chickpea. It has been reported from 35 countries (9), where it is especially major disease in West Asia, Nort Africa, South and East Europe, Northern Pakistan, and Nortwest India (10). Chickpea suffers from eight pathogens in Turkey (9), but Ascochyta blight is the most important one (11,12,13,14).

Although it is possible to control some stresses by the use of such inputs as agricultural chemicals, economic and environmental concerns limit their use in many farmers' field. Also, integrated management systems and agricultural inputs such as late spring-sown and cultural practices and use of fungicides, foliar sprays and seed dressing are the way to escape from Ascochyta blight, but they can greatly reduce the quantity and quality of chickpea product. Developing resistant cultivars is the best way of reducing the damage of Ascochyta blight. The objectives of this review were to determine sources of resistance and inheritance of resistance to ascochyta blight and also to evaluate the breeding strategies.

Ascochyta Bligh in Chickpea

The causal fungus

Ascochyta blight was first described in 1911 in the Nort-West Frontier Province of British India. It has been known that there are sexual and asexual forms of Ascochyta blight *Poma rabiei* (Pass.) Khune & J.N.Kapoor;= *Mycosphaerella rabiei* (Kovach.) or *Didymella rabiei* (Kovach.) v. Arx. and *Ascochyta rabiei* (Pass) Labr of chickpea, respectively (7). The pathogen attacks all-above ground part of the plant. When the source of inoculum is seedborn, the seedling, under favourable conditions, develops dark-brown lesions on the stem. When the inoculum source is airborne spores, the first simptoms of blight usually appear as small necrotic specks in the newly formed leaves. Lesions on the stems, petioles, leflets, poods and seeds, lesions are seen at Fig. 1 (7).

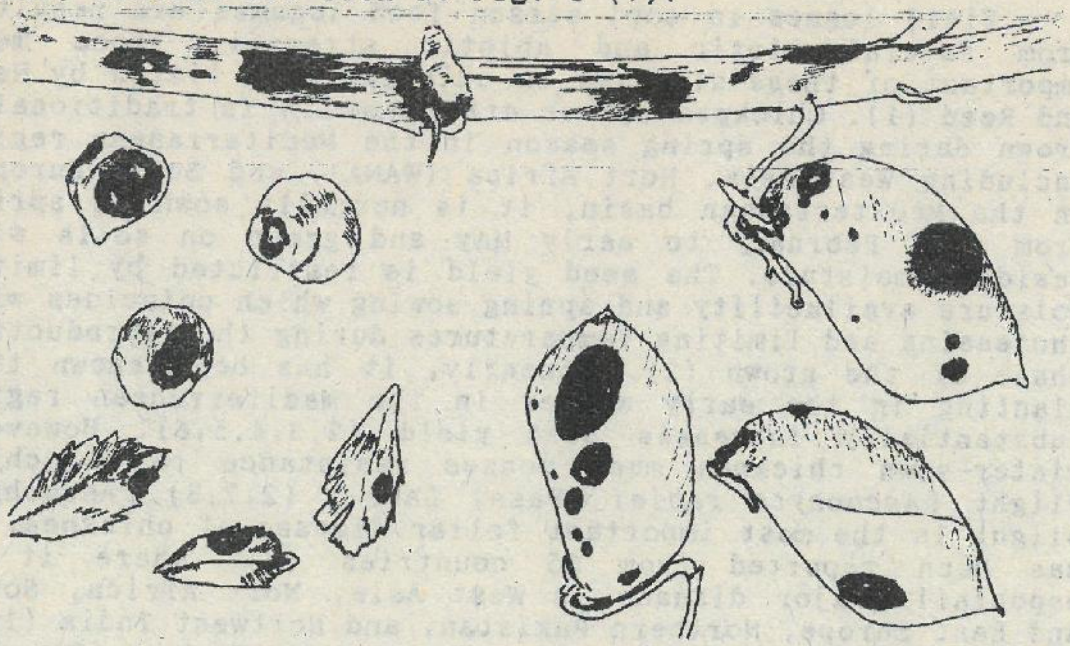


Figure 1. Lesions on the stems, petioles, leflets, poods and seeds (Nene and Reddy, 1987)

The asexsual or imperfect stage of the fungus is characterized by the formation of the fruting bodies (pycnidia) which produce spores (pycnidio spores or pycno spores). The pycnidia are visible as minute dots in the lesions on the host, either immersed or amphigenous, spherical or pear-shaped, with on ostiole, measuring up to 245 µ in diameter. In a transverse section of a pycnidium, the hyphae are hyaline to brown, and septate. The pycnidium contains numerous hyaline spores on sort conidiophores (stalk) embedded in a mucilaginous mass. When the pycnidia are wet, the mucilaginous mass absorbs moisture, swells and the spores ooze out. Pycnidispores are oval to oblong, straight or slightly bent at one or both ends, hyaline, occasinally bicelled but usually single celled measuring 8.2 to 10.0 x 4.2 to 4.5 µ (7).

It was first observed that the sexual stage of the fungus were *Mycosphorella rabiei* Kovachevski in 1936, in Bulgaria. The fruiting bodies, perithecia, were found exclusively on chickpea refuse, especially on pods that had overwintered in the field. They were dark brown or black, globose or aplanate, with a hardly perceptible beak and ostiole, varying in size from 76 to 152 x 120 µ. The asci were cylindrical-clavate, more or less curved, pedicellate and 48 to 70 x 13.7 µ in size. The ascospores (eight per ascus) were monostichous, rarely distichous, ovoid, divided into two unequal cells, strongly constricted at the septum and measured 12.5 to 19 x 6.7 µ (7). Since 1911, the disease has been reported from 35 countries (9) (Table 1).

Table 1. Countries from which Ascochyta Blight has been Reported.

Algeria, Australia,	Kenya,
Bangladesh, Bulgaria,	Lebanon, Libya,,
Canada, China, Colombia, Cyprus,	Mexico, Morocco,
Egypt, Ethiopia,	Pakistan, Portugal,
France,	Romania,
Greece,	Spain, Sudan, Syria,
Hungary,	Tanzania, Tunisia, Turkey
India, Iran, Iraq, Israel, Italy,	USA,
Jordan,	UIS (old USSR)

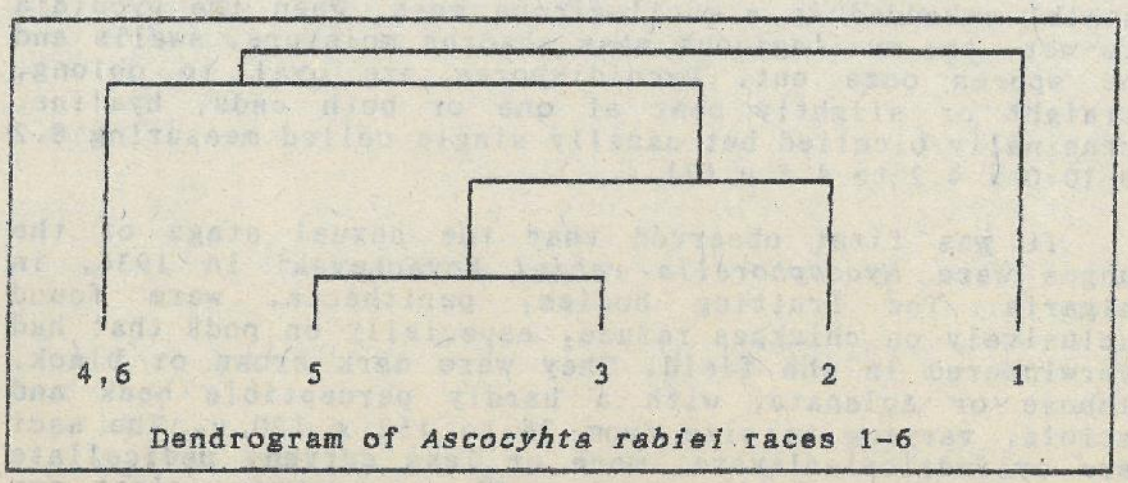
Source: Nene at.al., (1996).

Races of ascochyta blight

Work on physiologic races of *A. rabiei* has been intensified because of the serious losses caused by blight in recent years. The first awareness of race differences came from a report from India. In 1963, resistant cultivar C-12/34 against blight lost its resistance. In the later years, many of scientist studied variation in fungus isolates under controlled conditions on the basis of

symptomatology, manner of pycnidial formation on the host, and pathogenic behavior (7). They concluded that several races exist. Reddy and Siham (1984), reported six races of *A. rabiei* from Syria and Lebanon.

Recently, Oligonucleotide fingerprinting and DNA amplification fingerprinting, and the application of both programs by Kaemmer et.al. (15), resulted in the generation of very similar dendrograms (Fig. 2).



Source: Kaemmer et.al., (1992)

Figure 2. Relatedness of six *Ascochyta* isolates based on the comigration of fingerprint bands

Epidemiology

The fungus can survive for more than two years in naturally infected tissues at 10 to 35 °C and 0 to 3% relative humidity at the soil surface. However, the fungus lost viability within eight weeks at 65 to 100% relative humidity and at soil depths of 10 to 40 cm. The viability of fungus in diseased debris left over in the fields was lost within eight months and when buried 10 cm or deeper, it was lost within four months, in Syria (16). Maden et.al. (17) made a detailed study in Denmark of seed sample from Turkey. They found that 70% of seed from Central Anatolia was infected by *A. rabiei*. The inoculum occurred as spore contamination on the seed surface and mycelium and seed coat and embryo. Pycnidiospores from the seed surface and from pycnidia of 14-months-old seed stored at 3°±1 °C, showed 33% germination (7).

The spread of the disease has been attributed mainly to pycnidiospores produced at the foci of primary infection which occurs either though crop debris or infected seed. The disease spreads rapidly if wet and windy conditions occur in February and March when temperatures are around 22 to 26 °C. The incidence of blight was more than 50% during 15 years

that received, on average, more than 150 mm of rain. *A. rabiei* is racespesific, ie., most workers have reported *Cicer spp.* as the only host of *A. rabiei* (7).

Mechanism of resistance

Some workers considered that more malic acid secreted by leaves at flowering time favoured infection. In contrast, however, a resistant cultivar (F-8) secreted more malic acid than a susceptible cultivar (Pb-7), and that malic acid was inhibitory to spore germination and germtube development. It was found no difference in cuticle thickness between resistant and susceptible types, but found a greater acidity in the sap collected from resistant as compared with that from susceptible types. In compared biochemically a resistant cultivar (I-13) with a susceptible one (Pb-7) was found that the resistant cultivar showed higher peroxidase activity, higher L-cystine content, and higher phenolic compound content and higher catalase activity after inoculation (7).

These biochemical differences should explain the resistance. Also, lignin production in the plants might serve as an active defense mechanisim in the form of a mechanical barrier to prevent further spread of the pathogen in the host tissue (18). The hair of leaf and stem exudates and total amount of phenols did not affect the infection, but phytoalexins were considered to be one of the resistance factors (19).

Control

It was suggested that cultural practices such as the removal and destruction of dead plant debris, crop rotation, late spring-sowing and deep-sowing to prevent infected seeds from emerging should reduce the blight. In addition, sanitation, intercropping chickpea with such plants as wheat, barley and mustard could reduce disease spread in the crop season. The use of fungicides, foliar sprays and seed dressings are not usually advised because of reducing the quantity and quality of chickpea product. Under the above mentioned conditions, resistant cultivars are surely the best way against blight.

Sources of Resistance and Inheritance of Resistance

Many reports on identification of resistance to blight have appeared in the literature. In Turkey, the first study dealing with the inheritance of resistance, carried out by Eser (11), showed that a single dominant gene was responsible for resistance in line 72012. In addition, 36 lines, out of 5000 lines, were found resistant to varying degrees by Açıkgöz (20) in Turkey.

Singh and Reddy (22), studied that a total of 19.343 germplasm accessions of chickpea (12.748 desi and 6594 kabuli types) were evaluated for resistance to six races of *P.rabiei* at Tel Hadya, Syria, between 1979 and 1991. They found that only three desi accessions (ICC 4475, -6328 and -12004) and two kabuli accessions (ILC 200 and -6482) were resistant in repeated field and greenhouse evaluations. Also Source of resistance in cultigen to blight were identified between 1978 and 1993 by Singh (24), in ICARDA. These lines are; ILC 72, - 182, -187, -200, -2380, -2506, -2956, -3279, -3856, -4421, -5586, -5902, -5921, -6043, -6090, -6188 (24). Sources of resistance to blight identifed by several researcher are summurized in Table 2.

Table 2. Ascochyta Blight Resistant Lines

Genotypes	Inheritance of resistance	Reference
72012.....	A single dominant gene	Eser, 1976
ILC 191.....	A single recessive gene	Sing ve Reddy, 1983
ILC 200, ILC 201.....	A single dominant gene	Açıkgöz and Demir, 1984
ILC 195, Nec 138-1, 72012.....	A single recessive gene	Açıkgöz and Demir, 1984
ILC 72, -202, -2956, -3279.	A single dominant gene	Singh and Reddy, 1989
ILC 200, -6482.....		Reddy and Singh, 1992
ICC 4475, -6328, -12004.....		Singh and Reddy, 1993

Küsmenoglu et.al. (25) observed that no linkage was found between the gene for simple leaf (*svl*) and the loci Est-1, -2, -4, -5, Gal-1. However, they found that linkage occurred between the gene for plant growt habit (*hg*) and *Pgd-c* (phosphogluconate dehydrogenase), but ascochyta resistance and *Pgd-c* were not linked.

No single line resistant to both stresses, cold and blight, is available in the world gemplasm screening so far (26). Haq and Singh (2), found that resistant mutant line, ILC 482 Mut (M 17033), were scored 4 and 3 blight and cold, respectively. Sources of resistance in wild *Cicer* species for ascochyta blight and other important stresses are given in Table 3 (24).

Table 3. Sources of Multiple Resistance in Wild *Cicer spp.*

Accessions No.	*Cicer* species	Blight	Wilt	Leaf miner	Seed beetle	Cyst nematod	Cold	Drought
32	*bijugum*	S	R	S	R	R	R	S
62	*bijugum*	R	R	S	R	R	R	S
73	*bijugum*	R	R	S	R	R	R	S
79	*bijugum*	S	R	R	R	R	R	S
81	*reticulatum*	S	R	R	S	S	R	S
112	*reticulatum*	S	R	S	R	S	R	S
142	*reticulatum*	S	NE	S	NE	S	R	R
46	*judaicum*	S	R	R	R	S	S	S
158	judaicum	R	NE	NE	NE	NE	NE	NE
161	judaicum	R	NE	NE	NE	NE	NE	NE
163	judaicum	R	NE	NE	NE	NE	NE	NE
39	*echinospermum*	S	R	R	R	S	R	S
181	*echinospermum*	S	R	S	R	S	R	S
160	pinnatifidum	R	NE	NE	NE	NE	NE	NE
236	*pinnatifidum*	S	NE	R	NE	R	R	S

NE= Not evaluated, S= Susceptible, R= Resistant
Source: Singh, (1993).

Isolation and propagation

Ascochyta rabiei is easy to isolate and propagate. Suitable media and temperature and light requirements have been described. Many workers reported that pycnidia developed best at pH 7.6 to 8.6 at 20 ^{o}C on Ricard's medium of double concentration. Besides oatmeal agar, chickpea seed meal (4-8%) agar was also good medium. The fungus multiplies well on autoclaved chickpea seed. Also, chickpea dextrose broth (40 gr chickpea, 20 gr dextrose, 1 litre water) is a nice medium for large scale multiplication of fungus. The optimum temperature is around 20 ^{o}C. Temperatures below 10 ^{o}C are unfavourable to the fungus. Light affects growth of the fungus on artifical media, and continuous light increases sporulation (7).

Screening techniques

In 1931, the first an effort made was to identified resistance through artifical inoculation. In later years, the debris part of plant was used to infect on test plants. The best time to make artifically inoculations were flowering and podding periods.

Singh et.al. (27) was developed an efficient field screening procedure (Table 4). This involved; *(i)* simultaneous sowing of a row of a susceptible line after every 2-4 test rows; *(ii)* scattering debris collected in the previous season; *(iii)* maintaining high humidity through

sprinkler irrigation, and *(iv)* spraying plants with a spore suspention prepared from diseased plants, if required.

Table 4. Blight Severity in the Field

Score	Class	Blight severity
1.	immune reaction	no visible lesions on stems and leaves
2.	highly resistant	no lesions on stems, but lesions on leaves
3.	resistant	5% stems, leaves, and pods infected and stems broken, stem lesion 5 mm long, with few pycnidia
4.	moderately resistant	15% stems, leaves, and pods infected and stems broken, stem lesion 5 mm long, with few pycnidia
5.	tolerant	40% stems, leaves, and pods infected and stems broken, stem lesion 5 mm long, with more pycnidia
6.	moderately susceptiple	50% stems, leaves, and pods infected and stems broken, stem lesion 5 mm long, with more pycnidia
7.	susceptible	75% stems, leaves, and pods infected and stems broken, stem lesion 5 mm long, with more pycnidia
8.	highly susceptible	100% stems, leaves, and pods infected and stems broken, stem lesion 5 mm long, with more pycnidia
9.	very highly susceptible	all plants killed

Source: Singh et.al., (1981).

Reddy et.al., (28) used a glasshouse procedure for screening germplasm (Table 5). Ten seedlings of each germplasm line were grown in one pot. Two-week-old seedlings were inoculated by spraying them with an aqueous suspention of spores (20.000 spores ml^{-1}). Humidity was maintained by

covering the plants with plastic covers for 10 days. This method proved very useful for confirming field results (7).

Table 5. Blight Severity in the Greenhouse and Growth Chamber

Disease Rating	Reaction category	Buds killed %	Foliage infected %	Stems with lesions %	Stems broken %	Stem lesions type	Leaf lesion type	Pods with lesions %
1	Highly Resistant (HR)	Nil	Nil	Nil	Nil			Nil
2	Highly Resistant-Resistant (HR-R)	Nil	1.0	Nil	Nil		Necrotic with no or very few pycnidia	Nil
3	Resistant	0-2.5	5.0	80.0	5.0	No lesions girdling	Necrotic with few pycnidia	5.0
4	Resistant-Tolerant (R-T)	0-5.0	20.0	80.0	15.0	2 mm long girdling	Necrotic with few pycnidia	15.0
5	Tolerant (T)	10.0	40.0	100.0	40.0	2 mm long girdling	Necrotic with large number of pycnidia	40.0
6	Tolerant-Susceptiple (T-S)	25.0	50.0	100.0	50.0	2 mm long girdling	Necrotic with large number of pycnidia	50.0
7	Susceptible (S)	40.0	75.0	100.0	75.0	75 % girdling	Necrotic with large number of pycnidia	
8	Susceptible-Highly Susceptiple (S-HS)	100.0	90.0	100.0	100.0	100 % girdling	Necrotic with large number of pycnidia	100.0
9	Highly susceptible (HS)	plants completely killed						100.0

Source: Reddy et.al., (1984).

A Rapid Screening Technique and Its Importance

Though high levels of resistance are not available in cultivated genotypes, hovewer, there are good level of resistance to ascochyta blight in wild relatives of Cicer (24). But the frequency of success in crossing these wild species with cultivated genotypes is very low. Therefore, it is not advisable to destroy the whole plant derived from wide hybridization by screening techniques, which may be useful for other agronomic traits. There was a need to develop a quick and reliable screening technique, where only a branch of the plant can be used to judge the resistance without destroying the entire plant. Therefore, a cut-twig method of screening for ascochyta blight resistance was given by Sharma et.al.,(29), particularly for use in wide hybridization programs.

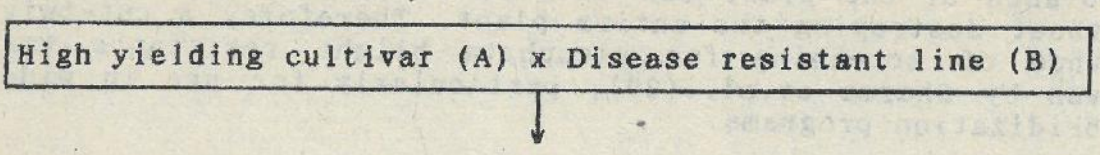
The technique consists of cutting 10-15 cm long tender shoots of chickpea plant by a sharp-edged razor during the evening. The cut-twigs are immediately immersed in water. Single twigs are wrapped with a cutton plug and transferred to a test tube (15 x 100 mm) containing fresh top water. These tubes are placed in a test tube stand and are inoculated by spraying spore suspention (40.000 spores ml^{-1}) of 14 days old culture of *A. rabiei*. The inoculated twigs are kept in moist-dasuti-cloth chambers for 72 h. There after, these inoculated twigs are moistened by spraying water by hand sprayer during the day from 1000 to 1600 h at 1 h intervals in order to provide uninterrupted leaf wetness and high relative humidity (above 90%) for 13 days. Disease symptoms appeared 6-8 days after inoculation, and there was 100% mortality in susceptible check and susceptible genotypes after 13 days of inoculation. The disease observations are recorded after 13 days of inoculation. This technique was compared with greenhous and field screening techniques and was found positively correlated (29).

Breeding Methods

All breeding methods applicable to self-pollinated crops are effective, provided genetic variation is present for the traits under consideration and the breeder can successfully separate this variability from the environmental variability for that traits. The breeding methods for self-pollinated crops are the pedigree, the bulk method and the various modified bulk methods, such as the mass pedigree method, the F_2-derived family method and the single-seed descent method (30). The breeding techniques are groupped; plant introduction and selection, hybridization, mutation breeding, cultivar mixture and different breeding methods by Singh (4).

Porta-Puglia et.al., (31) were explained; the stepwise breeding for multiple resistance using a single breeding programme (Fig. 3), the selection for resistance to ascochyta blight and fusarium wilt in alternate generations (Fig. 4) and the stepwise breeding for multiple resistance, using two parallel breeding programmes (Fig.5). Hovewer, many breeders have developed various modifications of one or more of these methods.

Also, the recurrent selection methods in self-pollinated plants have been summarized by Toker and Çağırgan (32). They suggested that the recurrent selection for seed yield and quality characters could successfully be used to self-pollinated crops.



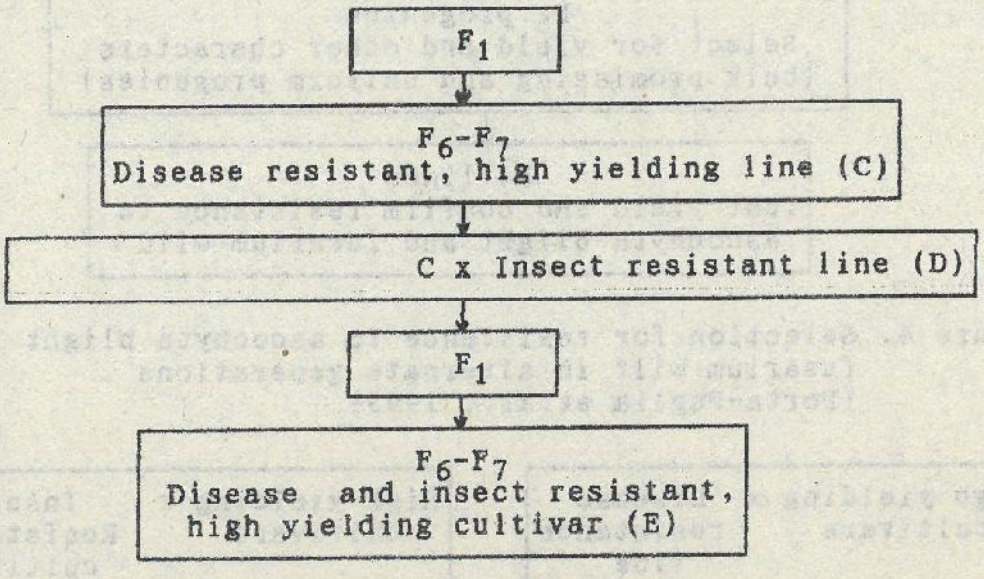
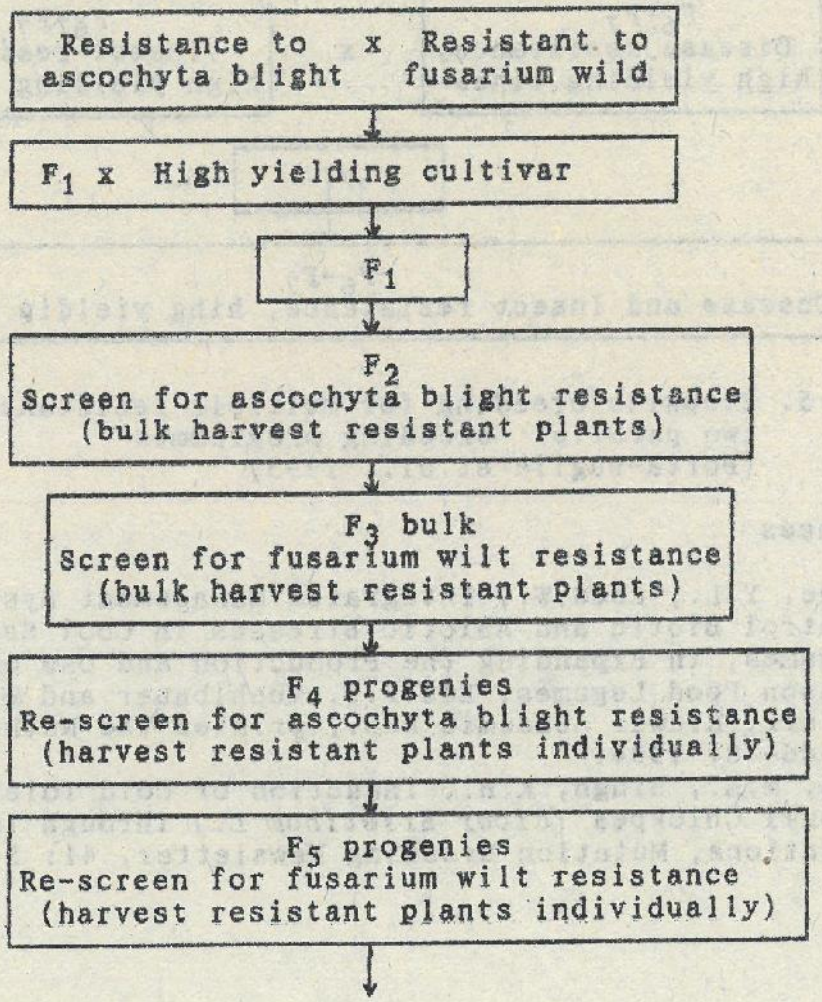


Figure 3. Stepwise breeding for multiple resistance using a single breeding programme (Porta-Puglia et.al., 1993)



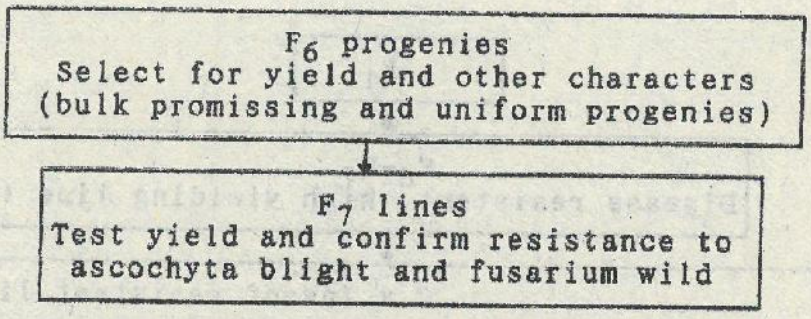


Figure 4. Selection for resistance to ascochyta blight and fusarium wilt in alternate generations (Porta-Puglia et.al., 1993)

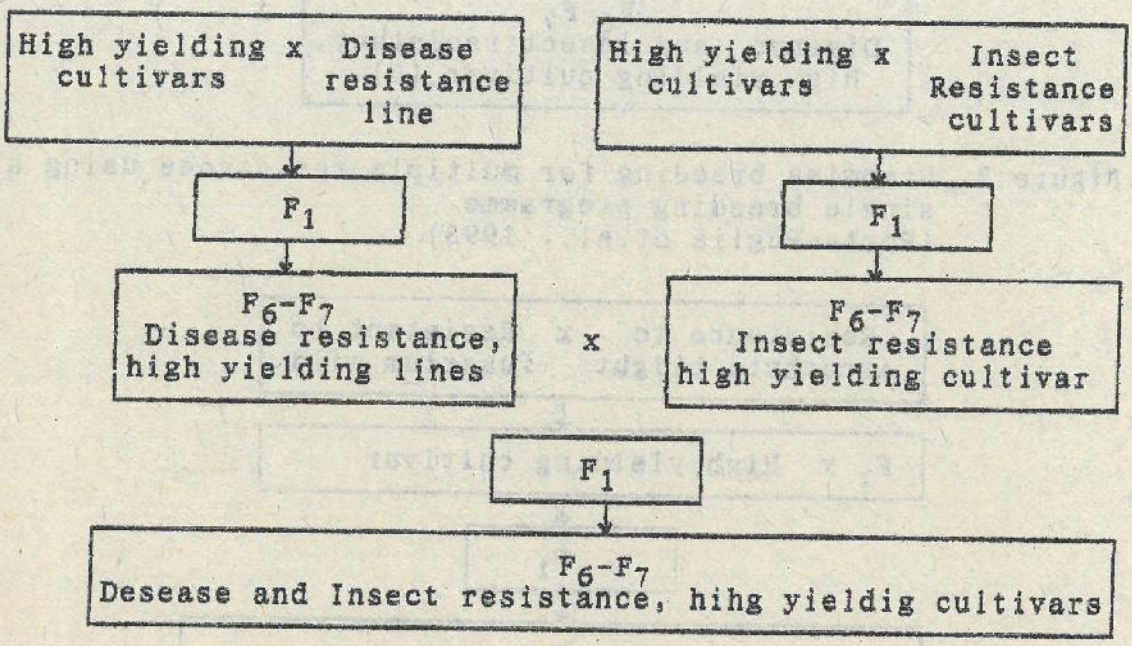


Figure 5. Stepwise breeding for multiple resistance, using two parallel breeding programmes (Porta-Puglia et.al., 1993)

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