LOCATING THE SOURCES OF RESISTANCE TO FUSARIUM WILT [*Fusarium oxysporum* f.sp. *niveum* : (E. F. Smith) Snyder and Hansen] IN WATERMELON (*Citrullus lanatus* Thunb.)

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Dedicated to

My Beloved Parents

and My Chairman

Dr. O. P. Dutta

DIVISION OF HORTICULTURE
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

CERTIFICATE

This is to certify that the thesis entitled **"LOCATING THE SOURCES OF RESISTANCE TO FUSARIUM WILT [*Fusarium oxysporum* f.sp. *niveum* (E.F.Smith) Snyder and Hansen] IN WATERMELON (*Citrullus lanatus* Thunb.)"** submitted by **B.P.SATHEESHA** for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **OLERICULTURE**, of the **University of Agricultural Sciences, Bangalore**, is a record of research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
January 9 ,1994

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(B.P.SATHEESHA)

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INTRODUCTION

I INTRODUCTION

Watermelon [*Citrullus* *lanatus*. (Thunb) Matsumura and Nakai] is a popular delicacy and an important vegetable throughout the plains of India. Watermelon has got many other vernacular names such as, Tarbooj, Kalinda, Jumika, Kalingarakaya, Kallangadi, Tharbuza and Palampanna. The genus *Citrullus*, is a small one containing only four species of which three belong to old world. Watermelon is indigenous to tropical Africa, more specifically South Africa (De Candolle, 1882).

Watermelon (2n=22) belongs to the family *Cucurbitaceae*. The plants are trailing or climbing hispid annuals with deeply or moderately divided leaves. Flowers are monocious and highly cross pollinated. Fruits are pepo. Fruits vary in size and shape. The flesh is soft and juicy and possess different shades of red, crimson and yellow or white colour. The seed colour may be white, brown, red, tan or black with two swellings on either side of the apex.

Small unripe watermelons are cooked as vegetable. Ripe fruits are used for dessert purpose. Fresh watermelon juce with salt and pepper is a popular cool and refreshing drink during summer. Its juice is useful to quenching the thurst and is also used as a drink with cool effect in types fever. In parts of Africa a yellow semi-drying oil is extracted from the seeds and is used in place of groundnut oil (Herklots, 1972).

Watermelon contains 93.1 per cent water. Per 100 gram of edible portion provides energy 31 calories, protein, 0.5 grams, calcium 7 mg, vitamin A 500 I.U., ascorbic acid 7 mg, thimene 0.069 mg, riboflavin 0.03 mg and niacin 0.56 mg.

The datas on area and production of watermelon is available only from five states (Assam, Gujrat, Punjab, U.P. and West Bengal). According to this data watermelon is being grown in an area of 16194 hectares with a production of 2,05,884 tonnes (Gill and Tomer, 1991).

Watermelon can be grown in almost all parts of India up to an elivation of 1524 mt. above mean sea level. It can be grown even in hot and dry regions. The main limiting factors in watermelon cultivation are diseases like Fusarium wilt, downy mildew, powdery mildew anthracnore, and virus, and insect pests like red pumpkin beetle, aphids, fruit fly, nematodes and thrips.

Fusarium wilt is a problem in cucurbits in general and in cultivated species of watermelon in particular. Most of the cultivated species of watermelon are susceptible to Fusarium wilt caused by *Fusarium oxysporum* f.sp. *niveum* (E.F. Smith) Snyder and Hansen Syn : *Fusarium niveum* E.F.Smith; *Fusarium bulbigenum* var *niveum*. The disease is known for more than 80 years. In India it was first reported in 1955 from Maharashtra (Bhide *et al*., 1955).

The plant is attacked in all stages of its growth.

Germinating seeds may rot in the soil. When very young seedlings are invaded, they may damp-off and die or be stunted in growth the cotyledons wilt, small leaves loose their green colour, droop and wilt. The hypocotyle is girdled by a watery soft rot. Characteristic wilt symptoms appear on old plants when leaves, show flagging down during hot periods of the day.

The wilting normally progress slowly. Initially only part of the plant may show symptoms but eventually the whole plant is affected. The pathogen is also responsible for necrotic lesions on the root system and development of vascular browning, gummosis and tylosis in the host xylem.

Fusarium oxysporum f.sp. niveum belongs to the class Deuteromycetes or fungi imperfecti whose sexual status could not be ascertained. Mycelium of this fungus is delicate, white or peach but usually with a purple tinge. Microconidia are generally abundent, variable, oval-elipsoidal or cylendrical. macroconidia are sparse, thin walled 3 septate and curved. The fungus is seed borne as well as persistant soil inhabitant. In presence of host, the chlamydospores of the fungus germinate and causes infection of the fibrus roots. The pathogen concentrates in the xylem vessels. Optimum temperature for growth of the fungus in culture is $27^{o}C$ (Singh, 1982).

Chemical control of such soil borne and root disease is very difficult. The disease can be checked to some extent by drenching the soil with captan or Hexocap or Thiride 0.2 - 0.3 %

solution. When chemical control of such a serious disease is not effective and economical, developement of resistant varieties is the only alternative to save the crop.

Many breeding programmes begining with Ortan (1907) has released watermelon cultivar resistant to Fusarium wilt. However, many of these have succumbed to wilt over the years due to the inherent ability of the fungus to form new races. At present there are three described races of *Fusarium oxysporum* f.sp. *niveum* race 0, 1 and 2 (Martyn, 1986). The most recently described one is race 2, first observed in Isreal in 1973 and in U.S.A. in 1981. Race 2 is highly aggressive and over comes all currently known wilt resistant genes. Since there is no watermelon variety resistant to Fusarium wilt developed in India, the present study was under taken at the division of vegetable crops, Indian Institute of Horticultural Research, (I.I.H.R) Hesaraghatta, Bangalore, with the following objectives.

1. To locate the sources of resistance to Fusarium wilt in watermelon germplasm collections.

2. To standardize a rapid screening technique for resistance to *Fusarium oxysporum* f.sp. *niveum* in watermelon.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Fusarium wilt is a serious problem in watermelon growing areas. Informations on sources of resistance for Fusarium wilt in watermelon cultivars would be most useful in planning and execution of breeding programmes to evolve a new variety or hybrid which is resistant to Fusarium wilt. Several studies have been reported on watermelon Fusarium wilt. Since the causal organism and symptoms are similar, a review of literature related to the present study from other members of the family Cucurbitaceae is summarised along with Citrullus lanatus. Thunb. under the following headings.

2.1 Causal organism/races

2.2 Method of inoculation

2.3 Sources of resistance

2.4 Genetics of resistance

2.5 Mechanism of resistance

2.1 CAUSAL ORGANISM

2.1.1 WATERMELON

Khodzhayan and Babayan (1963) found that Fusarium oxysporum f.sp. melonis isolated from muskmelon did not infected watermelon and cucumber in cross inoculation tests, where as Fusarium oxysporum f.sp. miveium, from watermelon and Fusarium aoxysporum f.sp. cucumerinum from cucumber, were strongly pathogenic to melons. Filtrates from cultures of f.sp. niveum and cucumerinum both caused wilt in melons and cucumber seedlings besides being toxic to original hosts.

Cruz et al. (1967) reported the presence of Fusarium species in the sandy soils of the high land region of Sao Paulo areas where the fungus has caused 10-15 per cent mortality in watermelon.

Armstrong and Armstrong (1978) studied the formae specials and races of Fusarium oxysporum causing wilt of Cucurbitaceae. They inoculated Fusarium oxysporum f.sp. cucumerinum, melonis, niveum, lagenariae and laffae to cucumber, muskmelon, watermelon and gourds. They reported that variation in virulence for a particular host occured but each formae speciales showed sufficient selective pathogenicity for the host from which it was derived to be retained as a valid formae speciales. Races were clearly defined for formae species melonis only.

Akhavizadegan (1983) isolated the fungus Fusarium oxysporum f.sp. niveum from infected plants in Guilan province of Iran and confirmed its pathogenicity by inoculating 20-30 days old seedlings. Chraleston Grey was more resistant to the fungus than other cultivars.

Nepa et al. (1985) studied the interaction of commercial watermelon cultivars with regional isolates of Fusarium oxysporum f.sp. niveum. Nine seed lots of 6 cultivars were root inoculated with four isolates from U.S.A. at the first leaf stage. They found that Calhoun Gray and Summit were not susceptible to the Edisto-2 (from south Carolina) and Leesburg-33 (from Florida) isolates but were highly susceptible to Texas isolate. The Texas isolate was most virulent. They concluded that the Texas isolate may be a different race of Fusarium oxysporum f.sp. niveum.

A highly aggressive race of Fusarium oxysporum f.sp. niveum was isolated in 1981 from wilted plants of Citrullus lanatus in South central Texas and in 1984 from north central Texas. In green house tests using 10^6 micro conidia/ml of isolates caused 87-100% wilt in 14 cultivars, 11 of which were considered highly resistant. The Texas isolates have been identified as race-2 (Martyn, 1986).

McMillan (1986) reported that isolates from wilted plants of cucumber or watermelon in Abaco Bahamas, were pathogenic to cucumber, watermelon and cantaloup melons regardless of original host. Strains of Fusarium oxysporum from wilted cucumber or watermelon plants from Florida were highly pathogenic to their original host species and are regarded as different formae species.

According to Mortyn and Burton (1989) Fusarium wilt is caused by a soil borne fungus Fusarium oxysporum Schletcht f.sp. niveum. The fungus has the inherent ability to form new races.

At present there are three described races of Fusarium oxysporum f.sp. niveum; race 0, 1 and 2. Race-2 is highly agressive and overcomes all currently known wilt resistant genes.

2.2 METHOD OF INOCULATION

2.2.1 WATERMELON

Beider (1977) developed a rapid method of evaluating watermelon varieties for resistance to Fusarium wilt. Wherein they measured phytoncide activity, accumulation of phenols, respiration rate and activity of oxidative enzymes under conditions of infection. All the above compounds increased to a greater extent in resistant variety than susceptible one Degeneration and lysis of the mycelium was more marked in the resistant variety.

Timchenkov and Beider (1979a) determined the resistance of watermelon vareities and hybrids on the basis of phytoncide activity, phenol content and peroxidase activity in 15 days old plants. The resistant variety had the highest phytoncide activity. Under conditions of infection, phenol content rose by 3-3.5 times in resistant variety but only 1.5-2 times in susceptible variety. Peroxidase activity increased more slowly in susceptible variety than in the resistant one.

Huang, et al. (1982) developed a method of screening watermelon cultivars in which they have grown 15 days old seedlings in filtrates from isolates of the fungus. Wilting occurred after 24 hours. They found that the filtrates were shown to contain a phytonivein like compound and thier virulence depended on the isolate from which they were obtained.

Simon and Galaev (1985) proposed a modified microhydroponic method of determining Fusarium wilt resistance in watermelon. Seedlings were inoculated at 1-2 true leaf stage and were grown in a microhydrophonic system. Disease severity was assessed 10 days after infection.

Wang and Zhang (1988) compared the soil root and root dip method of seedling inoculations to screen for Fusarium wilt resistance in watermelon. They found that root dip inoculation was fast, accurate and material saving. The optimum spore concentration was 5 x 10^3 spores/ml and root dip time 3 minutes.

Yu and Wang (1990) developed an appraisal method for assessing resistance to Fusarium wilt of watermelon, in which seedlings of 11 watermelon cultivars were soaked for 88 hours in a 50 per cent toxin solution containing mainly fusaric acid from liquid culture of *Fusarium oxysporum* f.sp. *niveum*. Peroxidase isozyme activity and banding pattern were determined before and after treatments. Peroxidase isozyme activity in the seedlings increased after soaking in the toxin filtrates. Seedlings of susceptible cultivar had 1 or 2 more isozyme bands than those of resistant cultivar.

Zhang et al., (1991) reported that root dipping was the best method for screening watermelon cultivars and the most suitable inoculum concentration being 1 x 10^5 to 1 x 10^6 micro conidia/ml.

2.2.2 MUSKMELON

Mas (1973) stated that in root dip method of inoculation the pathogen was capable of penentrating even the resistant varieties and surviving for up to three months in the lower parts of the plants without producing disease symptoms.

Soil inoculation with a micro conidial suspension resulted in only slight penentration of pathogen, but root dipping was followed by spread of the pathogen up to cotyledon level. In resistant plants microconidia survived atleast 3 months in hypocotyl but development above the first leaf was inhibited (Mas, 1973).

Sugahara (1974) reported that root dipping was the most effective method of screening muskmelon against Fusarium wilt and Selection 108, was the highly resistant source for Fusarium wilt.

McArdle and Kendall (1983) proposed a new method of inoculation for screening muskmelon cultivars to Fusarium wilt. The method involves (i) germinating two seeds in each Jiffy 7 peat pellet. (ii) Thinning the seedling to one (iii) inoculating each pellet with a 50 ml suspension containing 1,35,000 spores per ml of Fusarium oxysporum f.sp. melonis, when the seedlings reach the first leaf stage and score the seedlings 7 days after inoculation.

Radhakrishna and Sen (1985) compared efficacy of different methods of inoculation of Fusarium oxysporum f.sp. melonis and Fusarium solani for inducing wilt in muskmelon and reported that, fungi seed inoculation with fungi, always resulted in higher disease incidence than soil inoculation. The root dip treatment gave results highly consistant with those obtained from adult plant screening. This method is quicker and requires much less labour, material and bench space than the other two.

Megnegneou and Branchard (1988) treated the muskmelon callus initiated from cotyledon fragments on agar medium with different concentrations of fusaric acid (28 to 122 u.M) and selected the plantlets with tolerance to fusaric acid on the basis of callus texture, growth, rate of mortality and regeneration ability after one month in culture. The optimum concentrations of fusaric acid was 28 and 84 u.M.

2.2.3 CUCUMBER

Palmer and Willams (1981) evaluated cucumber seedlings for Fusarium wilt by following three methods, (i) sowing seeds directly in to sand infected with 10^5 spores/ml, (ii) Dipping roots of 7 days old seedlings in to inoculum containing 5 x 10^7 spores/ml and transplanting in to sand and (iii) inoculating as in (ii) but transplanting to sand infected with 10^5 spores/ml and found that sowing seeds directly in to sand infected with 10^5 spores/ml was the most efficient one.

Pelcz (1984) proposed a method of testing and assessing resistance to Fusarium wilt in cucumber which consists of immersion of seedlings for 10 minutes in a suspension of the fungus, followed by inoculation at 25°C. Disease assessment was carried out 24 days later.

Malepszy and El-Kazaz (1990) developed an *in vitro* method of selection of cucumber, resistant to *Fusarium oxysporum*. Callus and cell cultures of cultivar Broszczagowski and Gy-3 were challenged with different concentrations (1-5%) of cell free culture filtrates of *Fusarium oxysporum* f.sp. *cucumerinum* race OG-1 and FOCR-1. Resistant callus lines were isolated at the higher C.F.- concnetration and screened with 6 and 7 per cent C.F. Resistant cultures were again selected. Morphologically normal regenerated plants were obtained from selected callus and cell culture.

2.3 SOURCES OF RESISTANCE

2.3.1 WATERMELON

Bostos Çruz *et al*. (1968) reported that of 7 varieties evaluated in three glass house studies, Fair fax and *Charleston* Gray consistantly had a high degree of resistance or tolerance.

In Brazil only Fair fax and Charleston Gray showed a high tolerance to Fusarium wilt (Cruz, et al., 1968).

Michail et al. (1971) reported that watermelon variety Friska was the most resistant to Fusarium wilt in U.A.R.

Barnes (1972) reported that watermelon cultivars Calhoun Gray and Summit were highly resistant, Grahoma, New white Hope were moderately resistant, Hope Diamond, Charleston Gray, Shipher were slightly resistant and, Crimson sweet, ChrisCross, Jubilee and Black Diamond were susceptible to Fusarium wilt.

Tekanovich and Fursa (1975) studied the collection of the Soviet Institute of Plant Industry for resistance to Fusarium wilt. They found that cv. Klondike, Striped Blue Ribbon,Congo, Leesburg and Blacklee were highly resistant.

Paulus et al. (1976) tested 39 cultivars in 1-3 years for resistance to Fusarium wilt. Calahoun Gray showed the highest lewvel of resistance.

Mituskovic and Vucinic (1977) screened an unspecified number of varieties of watermelon for resistance to Fusarium oxysporum f.sp. niveum and found that Calhou Gray was highly resistant and Charleston Gray and Crimson sweet were moderately so. The susceptible varity Marmorka, when grafted on Lagenaria sp. remained free from infection even on highly infected soil.

Elmstorm and Crall (1979) bred a variety called Dixelee. The varieties involved in the ancestry were Texas W-5, Fair fax, Summit, Gray bell and wilt resistant Peacock 132. Fusarium wilt resistance in Dixelee was higher than in Crimson Sweet or Charleston Gray.

Crimson Sweet was resistant to Fusarium wilt. Some F_1 hybrids were partially resistant to Fusarium sp. (Barna, 1980).

Elmstorm and Hopkins (1981) reported that Calhoun Gray, Smokylee and Summit were highly resistant to Fusarium wilt and Sweet Princess, Jubilee, Charlesten-76, Klondike R 7, and Summer Field were slightly resistant.

Crall and Elmstorm (1981) developed a watermelon variety called Sugarlee. Sugarlee plants were moderately vigours, produced an early set of round, medium sized fruits with tough rind and high quality firm flesh. Sugarlee was resistant to anthrachose and has moderate resistance to Fusarium wilt.

Hopkins and Elmstorm (1983) studied the Fusarium wilt in watermelon cultivars grown in a 4 year monoculture. They observed an increase of Fusarium wilt in 10 cultivars from year to year except in Crimson Sweet. In the 4th year Crimson Sweet had the least wilt and the highest yield. They opined that Crimson Sweet appeared to have a unique type of resistance that was some what more stable in a monoculture than that of other cultivars.

Martyn and McLaughlin (1983) tested the effect of inoculum concentration on the apparent resistance of watermelon cultivars to Fusarium wilt. They found that cultivar resistance was dependent on the initial inoculum concentration. Resistance rankings for most cultivars usually dropped one level as inoculum concentration increased logarithmically. They observed that Dixelee and Smokylee remained highly resistant up to inoculum level of 1×10^6 conidia/ml.

Norton *et al*. (1985) developed two watermelon varieties, namely Au-Jubilent and Au-Producer. These two varieties are reistant to *Fusarium oxysporum* f.sp. *niveum*.

Netzer and Martyn (1989) reported that PI 296341 a breeding line of watermelon was highly resistant to race-2 of *Fusarium oxysporum* f.sp. *niveum*.

Mondal and Rashid (1990) observed that watermelon cultivars, Summit, Calhou Gray, Oasis and Royal Jubilee were highly resistant to Fusarium wilt.

2.3.2 **MUSKMELON**

Zink *et al*. (1982) reported that muskmelon cultivars Crenshaw, Earl's Favorite, Perlita, Small Persian, Snakemelon and Valley Gold were resistant to Fusarium wilt.

Ester and Gerlagh (1980) stated that muskmelon cultivars Haons, Ogen, Oranje, Ananas, Suiker Polidor, Overgen, Cantor, Cercon, Fusano, Pharo and Printadou were resistant to Fusarium wilt.

Kim et al. (1984) developed a new netted melon hybrid Poongmi by crossing Choonge 3 and Kurume 2. This variety was moderately resistant to Fusarium oxysporum f.sp. melonis.

Radhakrishna and Sen (1985) reported tht Indian cultivars Durgapur Madhu and Punjab Sunheri were resistant to both Fusarium oxysporum f.sp. melonis and Fusarium solani.

2.3.3 CUCUMBER

Jiang (1981) bred two cucumber hybrids, Ninghuang-1 (Jinyan 2 x Beijing Jietou) and Ninghuang-2 (Jinyan 2 x Chang Chun Mici). The hybrids were vigorous with higher resistance to downy mildew and Fusarium wilt than their parents.

2.4 GENETICS OF RESISTANCE

2.4.1 WATERMELON

Handerson et al. (1970) studied the inheritance of resistance to Fusarium oxysporum f.sp. niveum in the highly resistant Summit, moderately resistant Charleston Gray and susceptible variety New Hampshire Midget. They proposed 3 basic genetic models, a one locus multiple allele model and two, locus model. The multiple allelic model and the two locus model with the resistant parents having genes for resistance at different loci were both accepted as possible genetic models.

Henderson *et al.* (1970) suggested that a highly resistant variety such as Summit carrying a completely dominant source of resistance and could be used in obtainingF_1 hybrids and open pollinated varieties with good resistance to Fusarium wilt.

Netzer and Weintall (1980) studied the crosses between the susceptible variety Mallali and the resistant varieties Calhoun Gray and Summit, and stated that resistance to race-1 of *Fusarium oxysporum* f.sp. *niveum* was controlled by a single dominant gene.

Netzer (1982) studied the segregation data obtained in F_1, F_2 and back cross progenies of watermelon and suggested that resistance to *Fusarium oxysporum* f.sp. *niveum* race 0 and 1 was controlled by a single dominant gene. Based on the overall susceptibility to race-2, it was concluded that a non-specific polygenic resistance to this race, expressed much less in seedlings may be effective.

2.4.2 MUSKMELON

Risser (1973) studied the inheritance of resistance to race 1 and 2 of *Fusarium oxysporum* f.sp. *melonis* in the segregating line CM 17187 by artificial seedling inoculation. They found that resistance to both the races was dominant and monogenic (Fom 1,2). They also proved that this was independent of gene Fom 1 controlling resistance to race 1.

Zink et al. (1980) crossed Cucumis melo cultivar Perlita which was resistant to race-4 of Fusarium oxysporum f.sp. melonis, with susceptible PMR-45 and Top mark, and reported that the resistance was controlled by a single dominant gene.

Ester and Gerlagh (1981) reported that resistance to race 1,2 of Fusarium oxysporum f.sp. melonis in cv. Golden Pear was controlled by polygenes.

Zink et al. (1982) analysed the data from crosses of resistant Crenshaw, Earl's Favourite perlite, Small Persian, Snakemelon and Valley Gold with susceptible PMR-45, and reported that resistance to race 0 and 2 of Fusarium oxysporum f.sp. melonis was controlled by a single dominant gene.

The mode of inheritance of resistance to Fusarium oxysporum f.sp. melonis race 0 and 2 in cultivar Perlita FR and race 2 in Dublon was determined by analysing segregation of F_1, F_2 and BC_1 populations of the crosses with susceptible PMR-45. The ratios obtained indicated that resistance to both the races in Perlita FR was conferred by a single dominant gene. Allelism tests showed that gene Fom-1 for resistance in Dublon differed from the gene for resistance to race 0 in Perlita FR and that two different genes conferred resistance to race-2 in the two cultivars. Cross progenies of (resistant x susceptible) x susceptible inoculated simultaneously or sequentially with race 0 and 2 suggested that gene Fom-1 conferred resistance to race 0 and 2 in Dublon and that a single dominant gene in Perlita FR was designated as Fom-3. (Zink and Gubler, 1985).

Zink and Gubler (1986) studied the mode of inheritance of resistance to race 0 and 2 of Fusarium oxysporum f.sp. melonis in a gynoecious muskmelon line WI 1998 FR the segregation data of F_1, F_2 and BC_1 populations of the crosses with susceptible PMR-45 revealed that resistance to both races was conferred by a single dominant gene. Allelism tests indicated that the resistance was controlled by the gene Fom 1 which was different from the gene Fom-3 for resistance to these races in the cultivar Perlita FR.

Zink and Gubler (1986) reported that resistance in the cultivar U.C.PMR-45 to Fusarium oxysporum f. sp. melonis was conferred by the dominant gene Fom-1.

Zink and Thomas (1990) studied the genetics of resistance to Fusarium oxysporum f.sp. mel onis race 0, 1 and 2 in muskmelon line MR-1. Segregation ratios for F_2 and BC_1 populations of the crosses between resistant MR-1 and susceptible Top Mark indicated that resistance to race 1 and 2 of Fusarium wilt was conferred by single dominant gene. Linkage tests indicated that the genes for resistance to race 1 and 2 assort independently. Allelism tests showed that the single dominant in MR-1 was conferred resistance to race 0 and 2 and races 0 and 1.

2.4.3 CUCUMBER

Netzer *et al*. (1977) analysed the progenies of crosses between the resistant WIC 248 and susceptible shimshon. They found that resistance to Fusarium wilt was controlled by a dominant gene. They tentatively designated that gene as FOC.

2.5 MECHANISM OF RESISTANCE

2.5.1 WATERMELON

Vdovina and Belik (1974) observed that the fungitoxicity of the sap was increased more markedly in resistant varieties than in the susceptible ones after infection with *Fusarium oxysporum* f.sp. *niveum*.

Belik *et al*. (1974) reported that susceptible plants had weaker transpiration; their above ground organs contained less water than resistant plants. Resistant plants maintained or increased their transpiration rate after infection. The same was true for the content of minerals in their sap.

Timchenkov and Beider (1977) observed that dipping seeds in 50 per cent culture liquid before sowing reduced infection of watermelon plants by 10-15 per cent in the year of treatment. The increased resistance to wilt was connected with increased activity of oxidising enzyme.

Timchenkov and Beider (1979b) reported that the root system was better developed in the most resistant varieties, a broad layer of paranchymatous cells around the vascular bundles presented a barrier to the pathogen.

Mohammed et al. (1981) studied the susceptible cultivar Cango and resistant variety Citron of watermelon to Fusarium oxysporum f.sp. niveum and reported that resistance was due to a high level of preformed phenols which hindered infection, and to a phytoalexin (produced after infection) which prevented establishment of the pathogen with in the host tissue. The phytoalexin was produced at the expense of energy, released during respiration.

Wang and Zhang (1988) reported that the distribution of vessels, the number of central vessels in the root system and the thickness of the cell wall of the xylem determined the Fusarium wilt resistance in watermelon. There was no significant correlation between the activity of polyphenol oxidase or peroxidase and resistance but a strong correlation between viatmin C oxidase and resistance. There was no significant correlation between content of mallic acid or oxalic acid or total content of organic acid and Fusarium wilt resistance but a significant correlation between both acetic acid and citric acid and Fusarium wilt resistance. Resistant varieties had higher content of glycine, serine, alanine, threonine and arginine whereas susceptible varieties had higher contents of leucine methionine and tyrosine.

2.5.2 MUSKMELON

Kannaiyan and Purushothaman (1973) studied the correlation between cucurbitacin contents of the fruit and incidence of *Fusarium oxysporum* f.sp. *melonis*. They found that the resistant varieties like Smith's perfection and Dosakaya had high cucurbitacin content.

Netzer *et al*. (1979) reported that the increase in (1,3) glucanase activity following infection with *Fusarium oxysporum* f.sp. *melonis* race 0, was approximately twice as great in the root and hypocotyl of seedlings of resistant variety as in the seedlings of susceptible variety. They concluded that the increase in activity of (1,3) glucanase in the resistant variety was a part of the biochemical reaction responsible for the monogenically determined resistance.

Kesavan and Prasad (1974) reported that the less susceptible muskmelon cultivars contained higher content of cucurbitacin than more susceptible cultivars. A negative correlation was established between cucurbitacin content and the incidence of Fusarium wilt.

MATERIAL AND METHODS

III MATERIALS AND METHODS

The experiments pertaining to quick screening techniques and locating the sources of resistance to Fusarium wilt (Fusarium oxysporum f.sp. niveum) in watermelon (citrullus lanatus. Thunb) were conducted at the Division of Vegetable Crops, Indian Institute of Horticulture Research (I.I.H.R.), Hessaraghatta, Bangalore during the year 1992-93. The experimental site is situated at 13°58' north lattitude and 78° east longitude and at an elevation of 890 meters above mean sea level. Materials used and methods followed during the period of experimentation are described below.

3.1 MATERIALS

A total of 56 advanced breeding lines of watermelon were screened for their level of resistance to Fusarium wilt with Sugar Baby and Arka Manik varieties as susceptible checks. The seed material was supplied by the Division of Vegetable Crops I.I.H.R. Bangalore. The accession numbers and the sources of collection are given in the Table 1. The organic mixture (digested F.Y.M. + Neem cake) used during the present investigations was obtained from S.V. Rangaswamy and company, Bangalore.

Table 1 : List of watermelon accessions screened and their source of collection

Sl No.		Source of collection	Remarks
1	IIHR 118	Rajastan	A collection from Rajastan advanced for 3 generation
2	IIHR 51	Taiwan	Yellow Sweet(O.P) veriety
3	IIHR 40	U.S.A.	Crison Sweet selfed for 3 generation
4	IIHR 33	Japan	Festival Queen (F1) selfed for 6 generation
5	IIHR 138	Japan	Sugar Top (F1) selfed for 2 generations
6	IIHR 43	U.S.A.	Oasis (F1) selfed for 7 generations
7	IIHR 46	Japan	Summer Carnival (F1) selfed for 3 generations
8	IIHR 50	Durgapur	A collection from Rajastan
9	IIHR 45	U.S.A.	Paradise (F1) selfed for 6 generations
10	IIHR 119	Rajastan	A collection from Rajastan
11	IIHR 133	Bihar	A collection from Bhihar
12	IIHR 114	Rajastan	A collection from Rajastan
13	IIHR 20	U.S.A.	Cal Sweet (O.P.)
14	IIHR 35	U.S.A.	Sweet Meat (F1) selfed for 4 generations
15	IIHR 142	M.P.	A collection from Bhopal
16	IIHR 62	India	Milan (F1) selfed for 6 generations
17	IIHR 14	U.S.A.	Sugarlee (O.P.)
18	IIHR 24	U.S.A.	All Sweet (O.P.)
19	IIHR 17	U.S.A.	Petite Sweet (F1) selfed for 5 generations
20	IIHR 125	Haryana	A collection from Haryana
21	IIHR 121	Rajastan	A collection from Rajastan
22	IIHR 15	Japan	An introduction from Japan
23	IIHR 54	India	M.H.W. - 5 (F1) selfed for 5 generations
24	IIHR 11	U.S.A.	Juhla (F1) selfed for 5 generations

Contd...

Table 1 (Contd.)

Sl No.			Source of collection	Remarks
25	IIHR	136	Japan	World Champion (F1) selfed for one generation
26	IIHR	53	India	M.H.W. - 4 (F1) selfed for 6 generations
27	IIHR	107	Rajastan	A collection from Rajastan
28	IIHR	87	M.P.	A collection from Jabalpur
29	IIHR	36	U.S.A.	Peacock - 500 (O.P.)
30	IIHR	47	Japan	Summer King (F1) selfed for 5 generations
31	IIHR	140	Japan	Big Top (F1) selfed for 1 generation
32	IIHR	128	Haryana	A collection from Haryana
33	IIHR	63	I.I.H.R., Bangalore	Arka Joythi (F1) selfed for 5 generations
34	IIHR	152	Taiwan	Red Delicious (F1)
35	IIHR	32	Japan	Sweet Marvel (F1) selfed for 4 generations
36	IIHR	10	India	Giza (O.P.)
37	IIHR	39	U.S.A.	Sugar Baby
38	IIHR	34	U.S.A.	Royal Charleston (F1) selfed for 6 generations
39	IIHR	84	Japan	Petti Yellow
40	IIHR	3	A.P.	A collection from A.P.
41	IIHR	16	Japana	An introduction from Japan
42	IIHR	28	Japan	Cream Suika (F1) selfed for for 2 generations
43	IIHR	48	Japan	Pony Yellow (F1) selfed for 4 generations
44	IIHR	96	Rajastan	A collection from Udaipur
45	IIHR	86	Jabalpur	A collection from Jabalpur
46	IIHR	30	Japan	Sugar Delicata (F1) selfed for 5 generations
47	IIHR	122	Rajastan	A collection from Jaipur
48	IIHR	26	Japan	Honey Cream (F1) selfed for 4 generations
49	IIHR	13	U.S.A.	Dixilee (O.P.)

Contd...

Table 1 (Contd..)

Sl No.		Source of collection	Remarks
50	IIHR 37	Japan	Striped Sugar (F1) selfed for 6 generations
51	IIHR 25	Japan	Yellow Baby (F1) selfed for 5 generations
52	IIHR 5	U.P.	A collection from U.P.
53	IIHR 81	U.S.A.	Picnik (F1) selfed for 4 generations
54	IIHR 72	Japan	Fuken (O.P.)
55	Arka Manik	IIHR, Bangalore	A pure line
56	Sugar Baby	U.S.A.	A pure line

3.2 METHODS

3.2.1 COLLECTION, PURIFICATION AND MULTIPLICATION OF INOCULUM

Pathogenic isolates of Fusarium oxysporum f.sp. niveum were collected from the wilted watermelon plant grown at the experimental station of IIHR, Hessaraghatta, Bangalore. The infected roots were washed gently with running water and surface sterilized with sodium hypoclorite (2 %) for 30 seconds followed by three to four washings with distilled water. The roots were cut in to small pieces before transferring in to the potato dextrose agar (PDA) medium. The composition of the culture medium as suggested by Booth (1979) is detailed below:

Potato extract	: 250 ml
Dextrose	: 20 grams
Agar	: 15 grams
Distilled water	: Sufficient water to make up the volume to 1000 ml.

Potato extract was prepared by suspending 200 grams of pealed potato pieces in 50 ml of water and boiling it for 30 minutes. Twenty grams of dextrose and 15 grams of agar was suspended in 250 ml of potato extract and volume was raised to 1000 ml with distilled water. P.D.A. medium was autoclaved at 15 P.S.I. for 20 minutes. The sterilized P.D.A. medium was poured in to the sterilized petridishes under aseptic conditions and kept for solidification. Three to four pieces of surface sterilized watermelon roots, infested with Fusarium oxysporum f.sp. niveum were transferred in to each petridishes containing 5 ml of P.D.A. medium. The fungus was allowed to multiply on the medium for 7 days at 27°C in an incubator. Single spore of the above isolate was subcultured on the P.D.A. medium. Mass multiplication of the inoculum was done in the liquid broth culture. The composition of the liquid broth, as suggested by Booth (1979) is as follows:

Sucrose	: 20 g
Calcium nitrate	: 5.9 g
Potassium dihydrogen phosphate	: 1.1 g
Magnesium sulphate	: 0.4 g
Potassium chloride	: 1.6 g
Distilled water	: 1000 ml

A small piece of the pure culture was transferred in to the conical flask containing 100 ml of sterilized liquid broth under aseptic conditions and was mass multiplied at 27°C for 7 days in an incubator.

3.2.2 METHODS OF INOCULATIONS

The following three methods of inoculation were standardized for screening watermelon lines resistant to Fusarium wilt.

i. Seed treatment

ii. Seedling treatment

iii. Adult plant treatment

i. SEED TREATMENT

The seeds of susceptible watermelon variety Arka Manik were surface sterilized with 2 per cent sodium hypohlorite solution for 30 seconds followed by three to four washings with distilled water. The active inoculum containing micro and macro conidia of the pathogen grown on liquid broth culture medium was taken and its concentration was adjusted to 1 x 10^6 spores/ml using haemocytometer. Ten ml of the inoculum was poured in to autoclaved petridishes lined with two layers of germination paper and kept for fungal growth in an incubator at 27^o C for 7 days.

The following seed treatments were given for standardization of the screening technique at seed germination stage.

T_1 : Allowing the seeds and fungus to grow simultaneously in a petridish lined with germination paper.

T_2 : Allowing the surface sterilized seeds to germinate on the fungal mycelium already grown for 7 days in the petriplates lined with germination paper and soaked with 10 ml of fungal inoculum (1 x 10^6 spores/ml).

T_3 : Soaking the seeds in the fungal inoculum containing 1 x 10^6 spores/ml for 24 hour and placing the seeds on fungal mycelium already grown for 7 days in the petriplates.

T_4 : Soaking the seeds in the fungal inoculum (1 x 10^6 spres/ml) for 36 hour and placing the seeds on fungal mycelium already grown for 7 days in the petriplates.

T_5 : Soaking the seeds in the fungal inoculum (1 x 10^6 spores/ml) for 48 hour and placing the seeds on fungal mycelium already grown for 7 days in the peltriplates.

T_6 : Control : No seed treatment

Ten seeds of susceptible watermelon variety Arka Manik were used per replication in all treatments. The surface sterilized seeds were soaked in the fungal inoculum of concentration 1 x 10^6 spores/ml in a 100 ml beaker for desired duration. The soaked seeds were transferred into the petriplates in which fungus had already grown for 7 days under aseptic condition and kept in the incubator at 27°C for 7 days. The experiment was laidout in C.R.D. with 4 replications per treatment. Number of seeds germinated and survival percentages were calculated.

ii. SEEDLING TREATMENT

(a) STANDARDIZATION OF GROWING MEDIUM

In order to standardize a suitable medium for the simultaneous growth of the fungus and the seedlings, the following treatment were tried.

T_1 : Organic pot mixture + fungal inoculum

T_2 : Organic pot mixture + maize grains + fungal inoculum

T_3 : Organic pot mixture + barley grains + fungal inoculum

T_4 : Organic pot mixture + sorghum grains + fungal inoculum

T_5 : Control : Organic pot mixture without fungal inoculum

The grains and organic mixture were sterilized in a autoclave and mixed in a proportion of organic mixture : grain (5 : 1), and filled in polythene bags. Five ml of the fungal inoculum of concentration 1 x 10^6 spores/ml and 25 ml of liquid broth culture medium were added to each polythene bag. Polythene bags were kept in the incubator at 27^o C for 10 days for fungal growth. The experiment was laid out in C.R.D. with 4 replications. After 10 days, the fungal spore concentration was measured in each bag by taking 1 gram of medium in 10 ml of distilled water and counting the micro and macro conidia using hemacytometer.

(b) **STANDARDIZATION OF METHOD OF INOCULATION**

Seven days old seedlings of susceptile watermelon variety Sugar Baby growing in a petridish were taken. The roots were cut at a distance of 2mm from the tip and dipped in the fungal inoculum of concentration 1 x 10^6 spores/ml for 0, 0.5, 24, 36, 48 and 60 hours and kept in the incubator at 27^o C. Five seedlings per replication were transferred to polythene bags in which fungus was grown on a medium composed of sorghum grains + organic mixture, 7 days earlier. Twentyfive ml of the inoculum (1 x 10^6 spores/ml) was added to each polythene bag. After transplanting seedlings, the polythene bags were kept under shade for 2-3 days and then transferred to nylon net cage kept under the full sun light. The experiment was laidout in C.R.D. with 4 replications. The survival percentage was recorded 20-25 days after inoculation.

iii. **ADULT PLANT TREATMENT**

Watermelon variety Arka Manik was used for standardization of adult plant treatment. Seeds of Arka Manik were sown in the main field. When the plants were one month old, the following inoculation methods were tried.

T_1 : Injuring the stem at collar region with multiple pricks using a pin dipped in the inoculum of concentration 1 x 10^6 spores/ml.

T_2 : Inserting a cotton plug dipped in the inoculum (1 x 10^6 spores/ml) in to the collar region of the plants by making a slit with the help of blade.

T_3 : Pouring the fungal inoculum of concentration 1 x 10^6 spores/ml in to the root zone.

T_4 : Incorporating the fungus grown on the medium (Organic mixture + sorghum grains) for 7 days in to the root zone.

T_5 : Control : No treatment

The experiment was laid out in RBD with 4 replications keeping 5 plants per replication. Survival percentages were recorded 25-30 days after inoculation.

3.2.3 SCREENING WATERMELON GERMPLASM AGAINST RESISTANCE TO FUSARIUM WILT

Three method of screening were employed to evaluate watermelon germplasm reistant to Fusarium wilt.

(i) SCREENING AT SEED GERMINATION STAGE

A total of 56 accessions were screened using seed tretment method. Seeds of watermelon lines to be evaluated for their resistance to Fusarium wilt were surface sterilized with 2 per cent sodium hypoclorite for 30 seconds and thoroughly washed with distilled water two to three times. Twenty seeds of each accession were soaked with 10 m of the fungal inoculum of concentration 1 x 10^6 spores/ml in a beaker and kept in an incubator at 27^{o}C for 48 hours. The treated seeds were transferred to petriplates lined with germination paper on which fungus was grown seven days prior to seed placement. Five ml of the liquid broth medium was added to each plates under aseptic conditions and kept in an incubator at 27^o C for 5 days. The experiment was laidout in C.R.D. with two replications. Germination percentage was calculated 5 days after inoculation.

ii. SCREENING AT SEEDLING STAGE

One week old seedlings grown afresh as well as those which survived after the seed treatment were taken for conducting two separate experiments. The root tips were cut and dipped in the liquid broth inoculum of concentration 1 x 106 spores/ml for 48 hours and were transplanted in the polythene bags containing 200 grams of pot mixture and sorghum grains on which fungus was grown 7 days prior to transplanting. Twenty five ml of the above active inoculum was added in each polythene bag. Five seedlings per line per replications were transferred to each polythene bag. The polythene bags were kept under partial shade for two days and then transferred in to a nylon net cage kept in full sun light. The experiment was laid out in C.R.D. with two replications.

iii. SCREENING AT ADULT PLANT STAGE

One month old surviving seedlings of 25 lines from the root dip method as well as those grown afresh were transplanted to the main field in R.C.B.D. with two replication. While transplanting, the fungal inoculum grown on a standardized medium containing organic mixture + sorghum grain was spot incorporated at the base of seedlings. Recommended fertilizer dose and inter cultural operations were carried out for better establishment of the seedlings. A second inoculation was done 15 days after planting. Observations were recorded 25-30 days after inoculation. Tissue collected from wilted plants were examined under microscope for confirmation of the presence of the pathogen.

3.2.4 CONFIRMATION OF RESULTS

At the end of screening tests the resistant plants of each entry were selfed and the seeds were collected. The level of resistance of the selfed progenies was confirmed by using seed, and seedling inoculation methods described earlier.

3.2.5 MEASUREMENT OF INOCULUM CONCENTRATION

The concentration of fungal inoculum was determined with the aid of haemocytometer using the formula,

$$\text{Concentration (spores/ml)} = \frac{\text{Average No. of Conidia} \times 4 \times 10^{6}}{400}$$

3.2.5 PLANT DISEASE ASSESSMENT

The disease incidence/intensity was measured as the percentage of plants survived. The observations were recorded periodically. The number of dead seeds/seedlings were considered as susceptible and those which survived as resistant. The progenies were classified into following catagories.

Survival percentage	Reaction group
0 - 25	Highly susceptible
26 - 51	Susceptible
52 - 77	Resistant
78 - 100	Highly resistant

3.2.6 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The seed and seedling screening experiments were laid out in a replicated completly randomized design (CRD). Analysis of variance was derived by the standard statistical procedure and the survival percentage were compared taking in to consideration the CD, CV and SEM.

Adult plant screening was laid out in a replicated randomized complete block design (RCBD). The data was analysed as per standard statistical procedure. The data was interpreted using values of CD, CV and F test of significance (Sundararaj et al., 1972).

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

Experiments were conducted to standardise a quick screening techniques at seed, seedling and adult plant stage against Fusarium wilt in watermelon using watermelon varieties Arka Manik and Sugar Baby as susceptible checks. A total of 56 accessions including both indigenous and exotic collections were screened against *Fusarium oxysporum* f.sp. *niveum* using the best methods of inoculation at seed germination, seedling and adult plant stage. The results are presented below.

4.1 STANDARDIZATION OF A QUICK SCREENING TECHNIQUE

4.1.1 STANDARDIZATION OF SEED TREATMENT METHOD

Standardization of seed treatment method of screening was carried out in C.R.D. with 6 treatments and 4 replications. The observations were recorded 5 days after the treatment. Analysis of variance showed that treatment means pertaining to per cent germination differed significantly from each other (Table 2). The treatments T_4 and T_5 did not differ significantly from each other. The treatment T_4 (soaking the seeds in the fungal inoculum of concentration 1 x 10^5 spores/ml, for 36 hour before placing on the already grown fungal mycelium) gave only 10 per cent seed germination, whereas T_5 (soaking the seeds in the fungal inoculum for 48 hour before placing on the

Table 2 : Standardization of seed treatment method for screening watermelon against Fusarium wilt resistance under controlled conditions

	Treatments	Seed germination(%)
T1	Allowing the fungus and seed to grow simultaneously on the growing medium	60 a
T2	Placing the seeds on the fungal mycelium to germinate	45 b
T3	Seed soaking for 24 hr. in the inoculum and placing on fungal mycelium for germination	22.50 c
T4	Seed soaking for 36 hr. in the inoculum and placing on fungal mycelium for germination	10.00 d
T5	Seed soaking for 48 hr. in the inoculum and placing on fungal mycelium for germination	0.00 d
T6	Control(no seed treatment)	100.00 e

General mean	39.5833
F.Cal.	83.7100 **
S.Em.	4.0397
C.D. at 5%	12.1725
1%	16.8340

Values with same letter did not differ significantly each other at 5% C.D.

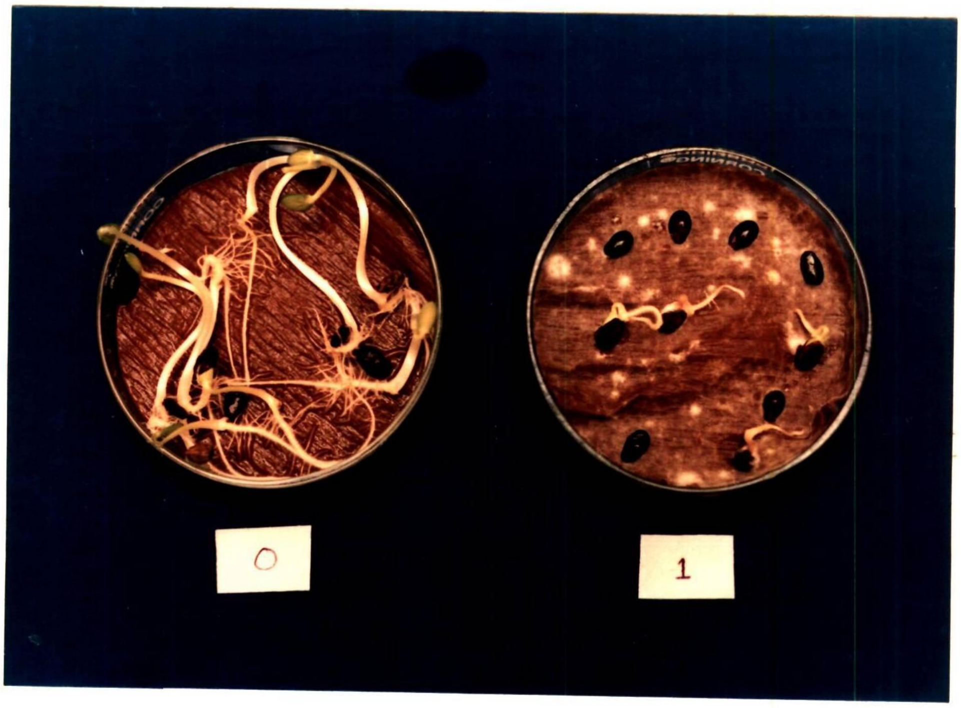
Plate 1 : Standardization of screening technique at seed germination stage

0 - No treatment

1 - Allowing the seeds to germinate on already grown fungal mycelium

Plate 2 : Standardization of screening technique at seed germination stage

1 - No treatment
2 - Seed soaking for 24 hour before placing on the fungal mycelium
3 - Seed soaking for 36 hour before placing on the fungal mycelium
4 - Seed soaking for 48 hour before placing on the fungal mycelium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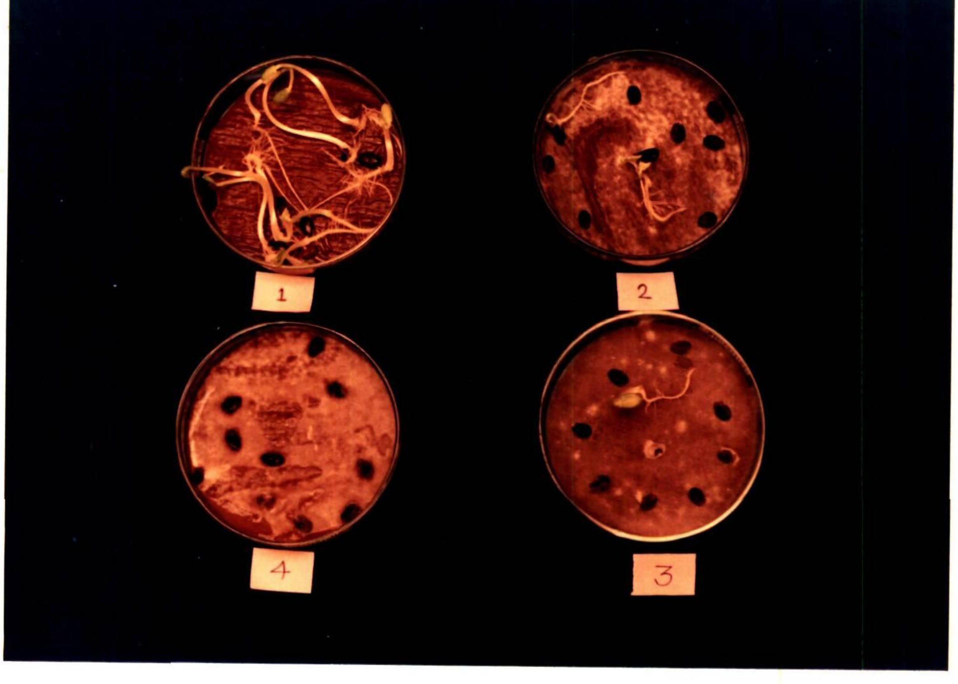
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fungal mycelium) totally inhibited the germination of the susceptible variety Arka Manik.

There was 100 per cent germination in untreated control (T_6) and germination of 60, 45 and 22.5 per cent was recorded in allowing the fungus and seed to grow simulteneously T_1), placing the seeds on already grown fungal mycelium (T_2) and soaking the seeds in the fungal inoculum for 24 hour before placing on the already grown fungal mycelium (T_3), respectively.

4.1.2 STANDARDIZATION OF MEDIUM FOR THE OPTIMUM GROWTH OF THE PATHOGEN IN POLY BAGS

For standardizing a suitable growing medium for the optimum development of Fusarium, 4 treatment combinations were tried with an untreated check. The experiment was laidout in C.R.D with 4 replications. The fungal spore population was measured 10 days after inoculation by taking 1 gram of sample in 10 ml of water. The results were expressed in number of spores/ml of water and are presented in Table 3.

Analysis of variance showed that treatments differed significantly from each other except for organic mixture + inoculum (T_1) and organic mixture + maize grains + inoculum (T_2). In organic mixture + inoculum the spore concentration was 7.25×10^4 whereas in organic mixture + maize, it was 18.5×10^4 spores/ml. The maximum number of spores (85×10^4) were observed in organic mixture + sorghum grain combination (T_4) followed by 55×10^4 spores/ml in organic mixture + Barley

Table 3 : Standardization of medium for optimum growth of *Fusarium oxysporum* f. sp. *niveum*

	Treatments	Mean concentration of spores (in 10000/ml of water)
T1	Organic mixture + inoculum (1×10^6 spores/m)	7.25 ab
T2	Organic mixture + maize seeds (5:1) + inoculum (1×10^6 spores/m)	18.50 b
T3	Organic mixture + Barley seeds (5:1) + inoculum (1×10^6 spore/m)	55.00 c
T4	Organic mixture + sorghum seeds (5:1) + inoculum (1×10^6 spore/m)	85.00 d
T5	Control: Organic mixture without inoculum	00.00 a

General mean	33.1500
F.Cal.	3.7188 **
S.Em.	93.1000
C.D. at 5%	11.4579
1%	16.0643

Values with the same letter did not differ significantly each other at 5% C.D.

grain combination (T_3). The treatment T_4 differed significantly from all other treatments.

4.1.3 STANDARDIZATION OF SCREENING TECHNIQUE AT SEEDLING STAGE

Standardization of screening technique at seedling stage was carried out with 7 treatments, using Sugar Baby as a susceptible check. The experiments was laid out in C.R.D with 4 replications. The roots of 7 days old seedlings were dipped in the fungal inoculum of concentration 1×10^6 spores/ml for different durations and transplanted into polythene bags containing a growing medium composed of O.M + sorghum grain on which the fungus was grown 7 days earlier. The number of wilted plants after 20 days of inoculation were counted and the mean per cent survival was taken to compare different treatments. The treatments differed significantly with each other. The results (Table 4) showed that root dipping in the inoculum of concentration 1×10^6 spore/ml for 48 hour (T_5) and for 60 hour (T_6) caused 100 per cent wilt in seedlings of susceptible variety Sugar Baby and differed significantly from all other treatments. Cent per cent survival was observed in untreated control (T_7) as well as in the treatment where roots were dipped in the inoculum for 30 minutes (T_1). Root dipping for 12 hour (T_2) recorded a survival of 85 per cent followed by 55 and 30 per cent in root dipped for 24 and 36 hour before transplanting to polythene bags.

Table 4 : Standardization of root dip method for screening watermelon germplasm against Fusarium wilt resistance

	Treatments	Mean survival of seedlings (%)
T1	Root dipping in inoculum for 30 min.	100 a
T2	Root dipping in inoculum for 12 hrs.	85 a
T3	Root dipping in inoculum for 24 hrs.	55
T4	Root dipping in inoculum for 36 hrs.	30
T5	Root dipping in inoculum for 48 hrs.	00 b
T6	Root dipping in inoculum for 60 hrs.	00 b
T7	Control (root dipping in water)	100 a

General mean	52.8571
F.Cal.	4.6291 **
S.Em.	90.1700
C.D. at 5%	13.7521
1%	18.8381

Values with the same letter did not differ significantly each other at 5% C.D.

4.1.4 STANDARDIZATION OF SCREENING TECHNIQUE AT ADULT PLANT STAGE

Standardization of adult plant inoculation method of screening was carried out with 5 treatments, taking Arka Manik as check variety. The experiment was laid out in R.C.B.D. with 4 replications. Per cent survival was taken as a criterion for comparing treatment means. The results of adult plant inoculation are presented in Table 5. Analysis of variance showed that incorporation of the fungus grown on a standardized medium composed of organic miture + Sorghum in to the root zone (T_4) induced 100 per cent wilt of the plants and differed significantly from all other treatments. There was 100 per cent survival of plants in untreated control (T_5) as well as in injuring the stem with multiple pricks made by a pin dipped in the fungal inoculum (T_1). This was followed by a 90 per cent survival in T_2 treatment in which a cotton plug dipped in the inoculum was inserted in to the collar region of the plants. Pouring the inoculum to the root zone gave a survival of 45 per cent in the field.

4.2 SCREENING WATERMELON ACCESSIONS FOR RESISTANCE TO FUSARIUM WILT

4.2.1 SCREENING AT SEED GERMINATION STAGE

A total of 56 accessions were screened by soaking the seeds in the inoculum of Fusarium oxysporum f.sp. niveum of concentration 1 x 10^6 spores/ml for 48 hour and placing on the

Table 5 : **Standardization of adult plant inoculation method of screening watermelon germplasm against Fusarium wilt resistance under field conditions**

	Treatments	Mean survival of plants (%)
T1	Injuring the stem with multiple pricks made by a pin dipped in the inoculum	100 a
T2	Inserting the cotton plug dipped in the inoculum into the coller region	90 a
T3	Pouring the inoculum into the root zone	45 b
T4	Incorporation of fungus grown on O.M + sorghum to the root zone	00 c
T5	Control : No treatment	100 a

General mean	67.0000
F.Cal.	3.6515 **
S.Em.	144.0000
C.D. at 5%	11.2506
1%	15.7737

Values with the same letter did not differ significantly each other at 5% C.D.

fungal mycelium grown on a germination paper inside the petriplates. The experiment was laidout in C.R.D. with two replications the germination count was taken 5 days after inoculation. The accessions were grouped as highly resistant, resistant, susceptible and highly susceptible according to their wilt percentage scale. Analysis of variance showed that the accessions differed significantly for their reaction to Fusarium wilt. Out of 56 accessions screened, 7 accessions (IIHR 118, 51, 119, 121, 40, 33 and 138) were found highly resistant and 4 accessions (IIHR 43, 46, 50 and 45) were found resistant to Fusarium wilt. Remaining 45 accessions were either susceptible or highly suscepible to the pathogen (Table 6).

Among the highly resistant accessions the germination varied from 80-100 per cent, considering the C.D. value at 1 per cent (12.48), the highly resistant accessions differed significantly from the resistant, susceptible and highly susceptible group. No significant differences in disease reaction were observed among the accessions within each reaction category except in the case of IIHR 118 which differed significantly in its reaction to Fusarium wilt from other accessions within the highly resistant group and accession IIHR 114 and IIHR 133 which differed significantly from 5 accessions (IIHR 142, 62, 14, 24 and 17) put under susceptible category.

4.2.2 SCREENING AT SEEDLING STAGE

Two experiments were conducted inorder to screen the

Table 6 : Screening watermelon accessions for resistance to Fusarium wilt using seed treatment method under controlled conditions

Lines	Survival percentage at seed germination stage	Reaction category
IIHR 118	100 a	HR
IIHR 51	85 b	HR
IIHR 119	80 bc	HR
IIHR 121	80 bc	HR
IIHR 40	80 bc	HR
IIHR 33	80 bc	HR
IIHR 138	80 bc	HR
IIHR 43	70 cd	R
IIHR 46	70 cd	R
IIHR 50	65 d	R
IIHR 45	60 de	R
IIHR 114	50 ef	S
IIHR 133	50 ef	S
IIHR 20	40 fg	S
IIHR 35	40 fg	S
IIHR 142	35 g	S
IIHR 62	35 g	S
IIHR 14	35 g	S
IIHR 24	30 g	S
IIHR 17	30 g	S
IIHR 125	15 h	HS
IIHR 15	15 h	HS
IIHR 54	10 hi	HS
IIHR 136	10 hi	HS
IIHR 53	10 hi	HS
IIHR 107	10 hi	HS
IIHR 87	10 hi	HS
IIHR 36	10 hi	HS
IIHR 47	10 hi	HS

Tbble 6 (Contd..)

Lines	Survival (germination) (%)	Reaction category
IIHR 140	05 hi	HS
IIHR 11	05 hi	HS
IIHR 63	05 hi	HS
IIHR 152	05 hi	HS
IIHR 32	05 hi	HS
IIHR 10	05 hi	HS
IIHR 128	05 hi	HS
IIHR 39	00 i	HS
IIHR 34	00 i	HS
IIHR 84	00 i	HS
IIHR 3	00 i	HS
IIHR 16	00 i	HS
IIHR 28	00 i	HS
IIHR 48	00 i	HS
IIHR 96	00 i	HS
IIHR 86	00 i	HS
IIHR 30	00 i	HS
IIHR 122	00 i	HS
IIHR 26	00 i	HS
IIHR 13	00 i	HS
IIHR 37	00 i	HS
IIHR 25	00 i	HS
IIHR 5	00 i	HS
IIHR 81	00 i	HS
IIHR 72	00 i	HS
Arka Manik	00 i	HS
Sugar baby	00 i	HS

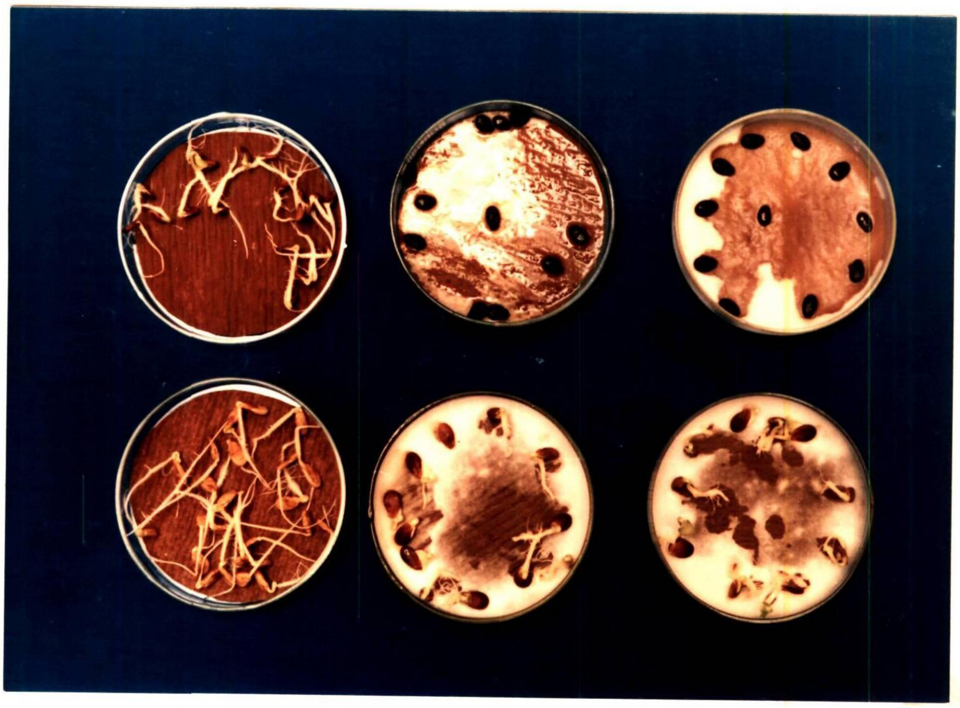
General mean	24.0000
F.Cal.	3.3029 **
S.Em.	85.0800
C.D. at 5%	9.3700
1%	12.4800

HR : Highly resistant
R : Reistant
S : Susceptible
HS : Highly susceptible

Values marked with same letter did not differ significantly from each other at 1 per cent C.D.

Plate 3 : Resistant Vs susceptible watermelon accessions when inoculated at seed germination stage

Top row : 1 : Control, 2&3 : Susceptible line Sugar Baby
Bottom row : 1 : Control, 2&3 : Highly resitant line I.I.H.R. 118



accessions for reistance to Fusarium wilt at seedling stage. In the first experiment the roots of the surviving seedlings of 25 accessions screened at seed germination stage were cut at a distance of 2 mm from the tip and dipped in the fungal inoculum of concentrtion 1 x 10^6 spores/ml for 48 hour and were transplanted in to the polythene bags in which the fungus was allowed to grow on a medium composed of organic mixture + Sorghum grown for a priod of 7 days. Seedlings of susceptible variety Sugar Baby were used as a check. The observations were recorded 20 days after inoculation. Analysis of variance showed that the accessions differed significantly in their reaction to Fusarium wilt (Table 7). Out of 25 accessions tested 21 accessions were highly resistant, one accession (IIHR 133) was resistant, 3 accessions (IIHR 54, 35 and 11) were susceptible and Sugar Baby was highly susceptible to Fusarium wilt. No significant difference in the reaction to Fusarium wilt was observed among the accessions within each category.

Within highly resistant group, the survival percentage varied from 80-100. Eleven accessions (IIHR 118, 51, 138, 46, 45, 20, 14, 142, 128, 136 and 53) had shown 100 per cent survival and differed significantly from resistant, susceptible and highly susceptible group.

The remaining 10 accessions of the highly resistant group (IIHR 33, 121, 50, 40, 119, 62, 43, 24, 17 and 114) showed seedling survival of 80-93.75 per cent. Considering the

Table 7 : Screening watermelon accessions for resistance to Fusarium wilt by giving root dip treatment to seedlings which survived seed treatment method of inoculation

Lines	Resistance (%)	Reaction category
IIHR 118	100.00 a	HR
IIHR 51	100.00 a	HR
IIHR 138	100.00 a	HR
IIHR 46	100.00 a	HR
IIHR 45	100.00 a	HR
IIHR 20	100.00 a	HR
IIHR 14	100.00 a	HR
IIHR 142	100.00 a	HR
IIHR 128	100.00 a	HR
IIHR 136	100.00 a	HR
IIHR 53	100.00 a	HR
IIHR 33	93.75 ab	HR
IIHR 121	93.75 ab	HR
IIHR 50	91.65 ab	HR
IIHR 40	87.50 ab	HR
IIHR 119	87.50 ab	HR
IIHR 62	87.50 ab	HR
IIHR 43	85.70 ab	HR
IIHR 24	83.33 ab	HR
IIHR 17	83.33 ab	HR
IIHR 114	80.00 ab	HR
IIHR 133	70.00 bc	R
IIHR 54	50.00 c	S
IIHR 35	50.00 c	S
IIHR 11	50.00 c	S
Sugar baby	-	HS

General mean	85.7580	HR	: Highly resistant
F.Cal.	10.3100 **	R	: Reistant
S.Em.	7.1397	S	: Susceptible
C.D. at 5%	20.8373	HS	: Highly susceptible
1%	28.2374		

Values marked with same letter did not differ significantly from each other at 1 per cent C.D.

C.D.Value, the accession IIHR 133 did not differ significantly from 10 accessions showing survival percentage ranging from 80-93.75, Highly susceptible accessions Sugar Baby could not survive at al.

In the second experiment freshly grown seedlings of watermelon accessions which showed more than 50 per cent survival during the screening at seed germiantion stage were given the root dip treatment as described earlier. Out of 15 accessions screened, 5 accessions (IIHR 118, 33, 51, 45 and 43) were highly resistant, 7 accessions (IIHR 46, 114, 119, 40, 35, 121 and 133) were resistant, 3 accessions (IIHR 138, 20 and 50) were susceptible and sugar Baby was highly susceptible to Fusarium wilt (Table 8). The accession IIHR 118 showed 100 per cent survival and differed significantly from all other groups. Four accessions (IIHR 33, 51, 45 and 43) of highly resistant group differed significantly from resistant susceptible and highly susceptible group except for 2 accessions (IIHR 46 and 114) classified unde resistant group. All the accessions of resistant group except IIHR 121 and IIHR 133 differed significantly from susceptible and highly susceptible group.

4.2.3 SCREENING AT ADULT PLANT STAGE

In the screening at adult plant stage two experiments were conducted. In the first experiment, the individual seedlings showing resistance to Fusarium wilt when inoculated at

Table 8 : Screening watermelon accessions grown afresh for resistance to Fusarium wilt using root dip treatment

Lines	Resistance (%)	Reaction category
IIHR 118	100.00 a	HR
IIHR 33	86.00 ab	HR
IIHR 51	80.00 bc	HR
IIHR 45	78.50 bc	HR
IIHR 43	78.38 bc	HR
IIHR 46	73.50 bcde	R
IIHR 114	70.00 bcde	R
IIHR 119	67.00 cdef	R
IIHR 40	62.50 cdef	R
IIHR 35	59.00 def	R
IIHR 121	54.50 ef	R
IIHR 133	54.05 ef	R
IIHR 138	50.00 fg	S
IIHR 50	34.00 gh	S
IIHR 20	30.00 gh	S
Sugar baby	00.00 i	HS

General mean	58.8234
F.Cal.	41.8200 **
S.Em.	4.2974
C.D. at 5%	12.9488
1%	17.9075

HR : Highly resistant
R : Reistant
S : Susceptible
HS : Highly susceptible

Values marked with same letter did not differ significantly from each other at 1 per cent C.D.

Plate 4 : Resistant Vs susceptible watermelon accessions when inoculated at adult plant stage

Left : Highly resistant line I.I.H.R. 118
Right : Highly susceptible line Sugar Baby


IIHR 118
SUGAR BABY

seed and seedling stage were transplanted to main field. While planting, the soil was inoculated with the fungus grown on the medium composed of organic mixture + Sorghum grains. A second inoculation was given to the root zone of each plant by adding fungal growth 15 days after first inoculation. The data presented in Table 9 showes that out of 25 accessions tested 20 accessions (IIHR 118, 119, 121, 51. 46. 138. 133. 14. 20. 142. 136, 128, 53, 33, 114, 40, 45, 62 and 24) were highly resistant, 2 accessions (IIHR 43 and 35) were resistant and 3 accessions (IIHR 17, 54 and 11) were highly susceptible to Fusarium wilt.

Thirteen highly resistant accessions showing 100 per cent survival (IIHR 118, 119, 121, 51, 46, 138, 133, 14, 20, 142, 136, 128 and 53) differed significantly from accessions put under resistant (IIHR 35) and highly susceptible group. Two accessions (IIHR 43 and 35) did not differ significantly from 7 accessions showing survival of 83.3 - 90 per cent.

Statistically there was no significant difference among the members within highly resistant, resistant and highly susceptible groups.

In the second experiment fresh seeds of watermelon accessions which showed survival percentage of 50 and more during the screening at seed germination stage were sown directly in the field and the adult plants of each accessions were inoculated as described earlier.

Table 9 : Screening watermelon accessions for resistance to Fusarium wilt by giving adult plant inoculation to plants which survived seed and seedling methods of inoculation

Lines	Resistance (%)	Reaction category
IIHR 118	100.00 a	HR
IIHR 119	100.00 a	HR
IIHR 121	100.00 a	HR
IIHR 51	100.00 a	HR
IIHR 46	100.00 a	HR
IIHR 138	100.00 a	HR
IIHR 133	100.00 a	HR
IIHR 14	100.00 a	HR
IIHR 20	100.00 a	HR
IIHR 142	100.00 a	HR
IIHR 136	100.00 a	HR
IIHR 128	100.00 a	HR
IIHR 53	100.00 a	HR
IIHR 50	90.00 ab	HR
IIHR 33	87.50 ab	HR
IIHR 114	87.50 ab	HR
IIHR 40	85.70 ab	HR
IIHR 45	83.33 ab	HR
IIHR 62	83.33 ab	HR
IIHR 24	83.33 ab	HR
IIHR 43	74.99 ab	R
IIHR 35	70.83 b	R
IIHR 17	00.00 c	HS
IIHR 54	00.00 c	HS
IIHR 11	00.00 c	HS
Sugar baby	00.00 c	HS

General mean	78.7110	HR	: Highly resistant
F.Cal.	30.7000 **	R	: Reistant
S.Em.	6.3705	S	: Susceptible
C.D. at 5%	18.5559	HS	: Highly susceptible
1%	25.1048		

Values marked with same letter did not differ significantly from each other at 1 per cent C.D.

Out of 14 accessions screened, 8 accessions (IIHR 118, 119, 121, 43, 133, 51, 35 and 50) were highly resistant, 6 accessions (IIHR 138, 45, 40, 46, 114 and 33) were resistant and Sugar Baby was highly susceptible to Fusarium wilt (Table 10). One accession (IIHR 118) of highly resistant groups differed significantly from resistant and highly susceptible group and showed 100 per cent survival to Fusarium wilt.

There was no significant difference among the accessions with in each category classified as highly resistant, resistant and highly susceptible.

4.2.4 CORRELATION STUDIES BETWEEN SCREENING AT SEED GERMINATION, SEEDLING AND ADULT PLANT STAGE

The results of the correlation analysis between the survival percentage of accessions at seed germination, seedling and adult plant stage (Table 11) showed that all the three methods of screening were positively correlated. Considering the correlation coefficient at 1 per cent (0.6226), survival percentage of accessions at seed germination stage was significantly correlated with seedling stage (0.7649) and adult plant stage (0.6295). The correlation between survival percentge at seedling stage and adult plant stage was positive but not significant (0.4940).

Table 10: Screening watermelon accessions for resistance to Fusarium wilt using adult plant inoculation methods

Lines	Resistance (%)	Reaction category
IIHR 118	100.00 a	HR
IIHR 119	91.67 abcde	HR
IIHR 121	87.50 abcdef	HR
IIHR 43	83.30 abcdef	HR
IIHR 133	80.67 abcdef	HR
IIHR 51	80.00 abcdef	HR
IIHR 35	79.20 abcdef	HR
IIHR 50	77.50 abcdef	HR
IIHR 138	73.20 bcdef	R
IIHR 45	70.00 cdef	R
IIHR 40	70.00 cdef	R
IIHR 46	67.50 cdef	R
IIHR 114	66.67 ef	R
IIHR 33	63.35 f	R
Sugar baby	00.00 g	HS

General mean	72.7037
F.Cal.	13.3100 **
S.Em.	6.1592
C.D. at 5%	18.6809
1%	25.9272

HR : Highly resistant
R : Reistant
S : Susceptible
HS : Highly susceptible

Values marked with same letter did not differ significantly from each other at 1 per cent C.D.

Table 11 : Correlation between survival percentages of accessions at seed, seedling and adult plant stages when screened against Fusarium wilt

Lines	Survival percentage		
	Seed treatment	Seedling treatment	Adult plant treatmnet
IIHR 118	100	100.00	100.00
IIHR 119	80	67.00	91.67
IIHR 121	80	54.50	87.50
IIHR 43	70	78.38	83.30
IIHR 133	50	54.05	80.67
IIHR 51	85	80.00	80.00
IIHR 35	40	59.00	79.20
IIHR 50	65	34.00	77.50
IIHR 138	80	50.00	73.20
IIHR 45	60	78.50	70.00
IIHR 40	80	62.50	70.00
IIHR 46	70	73.50	67.50
IIHR 114	50	70.00	66.67
IIHR 33	80	86.00	63.35

Calculated correlation coefficients
at 5% = 0.4973
at 1% = 0.6226

Observed correlation coefficients

(i) Between seed and seedling stage : 0.7649
(ii) Between seed and adult stage : 0.6295
(iii) Between seedling and adult stage : 0.4940

4.3 CONFIRMATION OF RESISTANCE TO FUSARIUM WILT LOCATED IN WATERMELON ACCESSIONS

A total of 15 selfed progenies of the plants selected previously from the resistant and highly resistant entries were further screened by the seed, and seedling method of inoculation. The results are described below.

4.3.1 SEED TREATMENT METHOD (SELFED PROGENIES)

In this experiment selfed progenies of 15 accessions were evaluated for resistance to Fusarium wilt using seed treatment method. Out of 15 accessions 4 accessions (IIHR 118, 119, 46 and 40) were highly resistant, 7 accessions (IIHR 51, 43, 33, 121, 114, 133 and 50) were resistant, 3 accessions (IIHR 45, 35 and 20) were susceptible and 1 accession (IIHR 138) and Sugar Baby were highly susceptible to Fusarium wilt (Table 12). The highly resistant accession IIHR 118 (100 % survival) differed significantly from all other accessions except IIHR 119 (survival 85 %). The highly resistant group differed significantly from resistant, susceptible and highly susceptible group except for 3 accessions (IIHR 51, 43 and 33) classified under resistant group. These three accessions (IIHR 51, 43 and 33) differed significantly from susceptible and highly susceptible group. The remaining 3 accessions (IIHR 121, 114 and 133) differed significantly from susceptible and highly susceptible group except for one accession (IIHR 45) classified under susceptible

Table 12 : Confirmation test for resistance to Fusarium wilt in selfed watermelon progenies using seed treatment method

Lines	Resistance	Reaction category
IIHR 118	100.00 a	HR
IIHR 119	85.00 ab	HR
IIHR 46	80.00 b	HR
IIHR 40	79.15 b	HR
IIHR 51	70.00 bcd	R
IIHR 43	70.00 bcd	R
IIHR 33	70.00 bcd	R
IIHR 121	60.00 cde	R
IIHR 114	60.00 cde	R
IIHR 133	60.00 cde	R
IIHR 50	55.00 def	R
IIHR 45	50.00 ef	S
IIHR 35	45.00 f	S
IIHR 20	40.00 fg	S
IIHR 138	25.00 g	HS
Sugar baby	00.00 h	HS

General mean	60.6100
F.Cal.	31.0200 **
S.Em.	4.4138
C.D. at 5%	13.3800
1%	18.5800

HR : Highly resistant
R : Reistant
S : Susceptible
HS : Highly susceptible

Values marked with same letter did not differ significantly from each other at 1 per cent C.D.

group. The susceptible accession IIHR 35 differed significantly from highly susceptible group. Sugar Baby was highly susceptible and showed 100 per cent wilting.

4.3.2 SEEDLING TREATMENT (ROOT DIP METHOD)

Freshly grown 7 days old seedlings of 15 accessions which had shown more than 50 per cent survival in the previous generation were further evaluated by the seedling treatment method in the polythene bags. Out of 15 accessions 3 accessions (IIHR 118, 119 and 40) were highly resistant, 5 accessions (IIHR 51, 133, 50, 114 and 33) were resistant, 6 accessions (IIHR 43, 121, 45, 35, 20 and 138) were susceptible and 1 accession (IIHR 46) and Sugar Baby were highly susceptible to Fusarium wilt (Table 13). One accession (IIHR 118) showed 100 per cent resistance and differed significantly from all other accessions. Other two accessions (IIHR 119 and 40) differed significantly from resistant, susceptible and highly susceptible group except for 3 accessions (IIHR 51, 133 and 50) classified under resistant group. Two accessions of resistant group (IIHR 51 and 133) differed significantly from susceptible and highly susceptible group.

No significant differences were observed among the members within resistant, susceptible and highly susceptible group. (Seedlings of 15 selfed progenies which survived the seed treatment method of inoculation were further inoculated by root dip method. Observation pertaining to disease reaction could not be recorded due to damage done by the rats at seedling stage).

Table 13 : Confirmation test for resistance to Fusarium wilt in selfed progenies of watermelon using root dip method of inoculation

Lines	Resistance	Reaction category
IIHR 118	100 a	HR
IIHR 119	80 b	HR
IIHR 40	80 b	HR
IIHR 51	75 bc	R
IIHR 133	70 bc	R
IIHR 50	65 bcd	R
IIHR 114	60 cd	R
IIHR 33	60 cd	R
IIHR 43	50 de	S
IIHR 121	50 de	S
IIHR 45	40 ef	S
IIHR 35	40 ef	S
IIHR 20	40 ef	S
IIHR 138	30 f	S
IIHR 46	00 g	HS
Sugar baby	00 g	HS

General mean	60.6100
F.Cal.	31.0200 **
S.Em.	4.4138
C.D. at 5%	13.3800
1%	18.5800

HR : Highly resistant
R : Reistant
S : Susceptible
HS : Highly susceptible

Values marked with same letter did not differ significantly from each other at 1 per cent C.D.

DISCUSSION

V DISCUSSION

Fusarium wilt of watermelon was first described in the South eastern United States by Erwin-F. Smith (1894). Watermelon wilt was first noticed in India in Dorli Village of Thana district of Maharashtra state in a serious form (Bhide *et al*., 1955). The pathogen *Fusarium oxysporum* f.sp. *niveum* (E.F.Smith) Snyder and Hansen was isolated from the roots of wilted watermelon plants in Bombay (Hegde and Bhide, 1958).

Jhamaria and Patel (1971) reported the presence of watermelon wilt both at the seedling and adult plant stage in Rajasthan during summer season. There are three known races of *Fusarium oxysporum* f.sp. *niveum* of which race 2 is highly aggressive, capable of causing 87-100 % wilt of watermelon (Martyn, 1986).

The present investigations which deal with the standardisation of quick screening techniques as well as screening for resistance to Fusarium wilt were carried out at I.I.H.R., Hessaraghatta, Bangalore. The findings of the investigations are discussed here under.

5.1 STANDARDIZATION OF QUICK SCREENING TECHNIQUES

Since the pathogen attacks the watermelon at seed germination, seedling and adult plant stages, it was desirable to standardise screening techniques at different stages of plant development.

5.1.1 STANDARDIZATION OF SEED TREATMENT METHOD

In standardization of screening technique atr seed germination stage, it was found that the treatments in which the seed and the fungus were allowed to grow simultaneously as well as those in which seeds were placed on already grown fungal mycelium were not conducive to screen watermelon accessions resistant to Fusarium wilt because such treatments could not completely kill the susceptible check variety at seed germination stage. Soaking the seeds in the fungal inoculum of concentration 1×10^6 spores/ml for 48 hour and placing the seeds on the already grown fungal mycelium on germination paper inside the petriplates and incubating at 27^oC for 5 days was found to be the best treatment which caused total death of susceptible check. Even seed soaking in the inoculum of concentration 1×10^6 spores/ml for 36 hour and placing on the fungal mycelium and incubating for 5 days at 27^oC could also be used as the next best treatment, since there was no significant difference between seed soaking for 36 hours and 48 hours.

Soaking seeds in the inoculum for 36 and 48 hours helped in the penentration of the fungal mycelium into the activated embryo, followed by profused fungal growth both with in as well as around the seed coat causing complete death of the seeds. By using this technique final results could be obtained just in 5 days.

5.1.2 STANDARDIZATION OF SEEDLING TREATMENT METHOD

a) standardization of a growing medium for optimum growth of the fungus:

From the results of this experiment, it was found that, when organic mixture alone was used as a medium, growth of the fungus was insufficient which led to the poor sporulation and low spore count per unit weight of the medium. The medium containing the organic mixture and sorghum grains in 5:1 proportion, increased the fungal growth and spore counts significantly when compared to a medium in which maize and barley grains were used. Incorporation of sorghum grains thus provided a medium for optimum fungal growth and sporulation, whereas the organic mixture provided the optimum condition for the growth of seedlings.

b) Standardization of screening method at seedling stage:

Results of standardization of root treatment at various intervals showed that by dipping the cut roots of 7 days old seedlings, in the fungal inoculum of concentration 1×10^6 spores/ml for 30 minutes and transplanting to a polythene bag containing a medium composed of organic mixture + sorghum grains on which fungus was allowed to grow for a period of 7 days, resulted in 100 per cent seedling survival even in the susceptible check, hence this treatment was not conducive to screen watermelon accessions for resistance to Fusarium wilt. The survival percentage of Sugar Baby, a susceptible check decreased as the root dipping duration increased from 12 hours to 60 hours.

A total wilt of Sugar Baby seedlings was obtained when roots were dipped for 48 hour or 60 hour. In 60 hour root dip treatment, root rotting was observed which was not the case with 48 hour root dip treatment. Hence, root dipping for 48 hour in the fungal inoculum of concentration 1 x 10^6 spores/ml and transplanting in to polythene bags in which fungus was already grown on a standardized medium composed of organic mixture and sorghum grains was considered to be the best treatment. Using this technique, the results could be achieved with in 20 days.

5.1.3 ADULT PLANT SCREENING

In adult plant inoculation, it was found that injuring the stem at collar region by multiple pricks with infested needle and inserting the cotton plug dipped in the inoculum of concentration 1 x 10^6 into the collar region of the plant were not effective in causing wilt of susceptible variety Arka Manik. Other two treatments like pouring the inoculum into the root zone and incorporating live inoculum grown on a medium composed of organic mixture and sorghum, in to the root zone caused considerable wilt of susceptible variety Arka Manik.

Incorportion of fungus grown on a medium (organic mixture + sorghum grains) in to the root zone when the plants are one month old caused 100 per cent wilt in susceptible check and could be used as a standard procedure for adult plant screening of watermelon accessions. The medium (organic mixture + sorghum grain) when added in to the soil helped in the establishment of the fungus near root zone and subsequent infection of roots by the fungus casusing higher percentage of wilt in the susceptible check. By using this method, results could be obtained in 55-60 days after sowing.

In conclusion we can say that seed screening i.e., soaking the seeds in the fungal inoculum of concentration 1×10^6 spores/ml for 48 hours and placing on the fungal mycelium grown on germination paper inside the petriplate followed by incubation for 5 days at 27^oC is the quickest screening technique for screening watermelon against Fusarium wilt which gave the results in 5 days compared to 20 days in seedling screening and 55-60 days in adult plant screening. This result is in contrary to that of Radhakrishna and Sen (1985) who reported that root dip method of screening was quicker method than seed and adult plant screening. The contrary result of present experiment might be due to the modifications made in the seed treatment over the traditional seed treatment method in which soaked seeds were directly sown in the field for assessing the disease reaction.

5.2 SCREENING WATERMELON ACCESSIONS FOR RESISTANCE TO FUSARIUM WILT

In the preliminary screening of watermelon accessions for resistance to Fusarium wilt at seed stage, seeds were soaked in the fungal inoculum of concentration 1 x 10^6 spores/ml for 48 hour and were placed on the already grown fungal mycelium inside the petridish. The manifestation of wilt reaction of the virulent pathogen was observed with in 5 days of inoculation resulting in the death of viable seeds of susceptible genotype.

Two simultaneous experiments were conducted to screen watermelon accessions at seedling stage. In the first experiment, the surviving seedlings after the seed treatment were root dipped in the fungal inoculum of concentration 1 x 10^6 spores/ml for 48 hour and transplanted into the polythene bag in which fungus was already grown on a standardized medium composed of organic mixture and sorghum grains. In each of the polythene bags 25 ml of inoculum of concentration 1 x 10^6 spores/ml was added after transplanting. This treatment helped in maintaining uniformity and optimum strength of inoculum with in the bag and gave consistant and reproduceable results. Resistance is known to be affected by inoculum density and inoculation method as suggested by Martyn and McLanghlin (1983). Since root dip treatment at seedling stage was applied only to those seedlings which could survive the previous treatments of inoculation at seed stage, it helped in enhancing the survival percentage at latter stages. In the second experiment, fresh seedlings of watermelon accessions which showed more than 50 per cent survival at seed stage screening were given the root dip treatment.

For screening watermelon accessions at adult plant stage, two experiments were conducted. In the first experiment, the watermelon accessions which survived at seed germination and seedling stage screening were inoculated at adult plant stage by incorporating the fungal growth to the root zone. In the second experiment, freshly sown plants of watermelon accessions which showed a survival of more than 50 per cent (at seed germination stage treatment) were inoculated at adult plant stage.

In order to confirm the level of resistance in different accessions, the resistant plants were selfed and their progenies were re-tested by screening at seed and seedling stages.

During the preliminary screening at seed germination stage, a total of 56 accessions were screened against resistance to Fusarium wilt (Fusarium oxysporum f.sp. niveum). In this experiment 80.36 per cent (45 accessions out of 56 accessions) of the watermelon accessions were found either susceptible or highly susceptible (0-51 per cent survival) and only 19.64 per cent (11 out of 56 accessions) were either resistant or highly resistant to Fusarium wilt (53-100 % survivals). One accession (I.I.H.R-118) showed 100 per cent survival to Fusarium wilt. This high degree of resistance might be due to the increased rate of respiration, phenolic content and enhanced activity of oxidative enzymes which may cause degeneration and lysis of mycelium as reported by Beider (1977) and Timchenko and Beider (1977).

During screening at seedling stage in the first experiment, 88 per cent (22 accessions out of 25 accessions) of the accessions showed either resistant or highly resistant reaction towards Fusarium wilt. Only 3 accessions (12 %) showed susceptibility to the pathogen. During screening at seed germination stage the survival percentage in different accessions varied from 5-100 per cent, whereas at seedling stage, the level of resistance ranged from 50-100 per cent. In two accessions (I.I.H.R.-53 and 136) the survival percentage increased from 10 pr cent in seed stage to 100 per cent in seedling stage i.e., an increase of 900 per cent from seed to seedling stage. The accession (IIHR 118) showed consistent expression of 100 per cent survival both at seed germination and seedling stages (Table 14).

In the second experiment freshly grown seedlings of 15 accessions which showed more than 50 per cent survival during screening at seed stage were inoculated with known concentration of inoculum. Eighty per cent (12 out of 15 accessions) accessions showed either resistant or highly resistant reaction towards Fusarium wilt. One accession (IIHR 118) showed a 100 per cent survival at seedling stage. Seven accessions (IIHR 33, 45, 43, 46, 114, 35 and 133) showed a higher level of survival

Table 14 : Survival percentage of watermelon accessions under continuous pressure of selection for resistance to Fusarium wilt

Lines	Survival percentage of plants		
	Seed stage	Seedling stage	Adult plant stage
IIHR 118	100	100.00	100.00
IIHR 119	80	87.50	100.00
IIHR 121	80	93.75	100.00
IIHR 51	85	100.00	100.00
IIHR 46	70	100.00	100.00
IIHR 138	80	100.00	100.00
IIHR 133	50	70.00	100.00
IIHR 14	35	100.00	100.00
IIHR 20	40	100.00	100.00
IIHR 142	35	100.00	100.00
IIHR 136	10	100.00	100.00
IIHR 128	05	100.00	100.00
IIHR 53	10	100.00	100.00
IIHR 50	65	91.65	90.00
IIHR 33	80	93.75	87.50
IIHR 114	50	80.00	87.50
IIHR 40	80	87.50	85.70
IIHR 45	60	100.00	83.33
IIHR 62	35	87.50	83.33
IIHR 24	30	83.33	83.33
IIHR 43	70	85.70	74.99
IIHR 35	40	50.00	70.83
IIHR 17	30	83.33	00.00
IIHR 54	10	50.00	00.00
IIHR 11	05	50.00	00.00
Sugar baby	00	00.00	00.00

percentage at seedling stage (59-86.6) when compared to survival at seed stage (40-80). The increase in the level of resistance at seedling stage was 47.5 per cent in accession IIHR 35 and 40 per cent in accession IIHR 114 when compared with the level of resistance observed at seed germination stage (TAble 8).

In contrary to this, 7 accessions (IIHR 51, 119, 40, 121, 138, 50 and 20) showed a reduction in their survival percentage at seedling stage when compared to those at seed stage. IIHR 50 showed a reduction of 47.6 per cent (65 % to 34%) followed by 37.5 per cent (80-50 %) in IIHR 138. These contradictory results might be due to the difference in the genetic architecture of the accessions as well as their mechanism of resistance to Fusarium wilt.

Since only those seedlings which could survive the treatment given at seed germination stage were selected for the screening at seedling stage, a certain degree of selection pressure is involved. This selection pressure, while screening at seed germination stage varied in 15 test lines. The selection pressure was 40 per cent in the line IIHR 20 and it was 100 per cent in the accession IIHR 118.

When the results of screening at seedling stage, with and without selection pressure at seed germination stage were compared, it was found that screening with selection pressure increased the survival percentage in all most all the accessions (Table 15). Further with the selection pressure at seed stage

Table 15 : Results of screening watermelon accessions with continuous selection pressure and without selection pressure at seedling stage

Lines	Per cent survival		
	Seed germination stage	With selection pressure applied at seed stage	Without selection pressure applied seed stage
IIHR 118	100	100.00	100.00
IIHR 33	80	93.75	86.00
IIHR 51	85	100.00	80.00
IIHR 45	60	100.00	78.50
IIHR 43	70	85.70	78.38
IIHR 46	70	100.00	73.50
IIHR 114	50	80.00	70.00
IIHR 119	80	87.50	67.00
IIHR 40	80	87.50	62.50
IIHR 35	40	50.00	59.00
IIHR 121	80	93.75	54.50
IIHR 133	50	70.00	54.05
IIHR 138	80	100.00	50.00
IIHR 50	65	91.65	34.00
IIHR 20	40	100.00	30.00
Sugar Baby		00.00	00.00

86.66 per cent of the accessions showed higher level of resistance to Fusarium wilt at seedling stage where as only 33.33 per cent of the accessions showed high level of resistance to Fusarium wilt in the absence of selection pressure at seed germination stage.

The reistance expressed at seedling stage could be due to the well developed root system with broad layer of parenchymatus cells surrounding the vascular bundle and presenting a barrier to the entry of the pathogen (Timchenkov and Beider 1979). In addition to this well distributed vessels, high number of central vessels in the root system and thick walls of the xylem might be responsible for the expression of resistance reaction (Wang and Zang, 1988).

During the screening of watermelon accessions at adult plant stage, 22 out of 25 accessions (88 %) showed highly resistant to resistant reaction (70.83-100% survival) to Fusarium wilt (Table 14). During the first experiment only 3 accessions (12 %) showed total wilting and got eleminated from the population. Continuous selection pressure applied at seed germination stage (5-100%) and seedling stage (50-100) helped in enhancing the survival per cent at adult stage of the plant. The survival per cent which was in the range of 5-100 when screened at seed germination stage was increased to 50-100 when screened at seedling stage and it was as high as 70.83 - 100 when screened at adult plant stage. In the adult plant stage 80 per cent of the accessions tested were highly resistant (83.33-100 %) as against 12.5 per cent (7 out of 56 accessions) at seed germination stage. Number of accessions which showed 100 per cent survival when screened at seed germination stage was only one where as screening at seedling stage 11 accessions showed 100 per cent survival, however at adult stage screening this number was enhanced to 13. Two accessions (IIHR 136 and 53) which showed only 10 per cent survival at seed germination stage however showed 100 per cent survival at adult plant stage following a selection pressure (10 %) at seed germination stage.

In the second experiment when freshly sown plants were inoculated at adult plant stage, all the 14 accessions showed either resistant or highly resistant reaction towards Fusarium wilt. The survival percentage in the population varied from 63.35 - 100. Eight accessions (IIHR 118, 119, 121, 43, 133, 51, 35 and 50) showed resistant or highly resistant reactions towards the pathogen. The range of survival percentage was the highest (63.35 - 100) at adult plant stage followed by at seed germination stage (40-100) and at seedling stage (30-100). The higher level of resistance to Fusarium wilt at adult stage could be due to the ability of the resistant accessions to increase their transpiration rate and mineral content in their sap after infection as suggested by Belik _et al_. (1974). It could also be due to the higher cucurbitacin content in the plant sap as reported by Kesavan and Prasad (1974) in musk melon.

When the results of screening at adult plant stage with the application of selection pressure and with out selection pressure were compared (Table 16), it was found that screening with selection pressure at seed and seedling stage resulted in higher survival percentage of plants compared to those with out the application of selection pressure. Eighty five per cent of the population exhibited high level of resistance (87.5-100 %) at adult plant stage with selection pressure as compared to 57.14 per cent of population showing high level of resistance (77.5 - 100 %) without selection pressure. With selection pressure, 7 accessions (IIHR 118, 119, 121, 133, 51, 138 and 46) showed a 100 per cent survival at adult plant stage as compared to only one accession (IIHR 118) showing 100 per cent survival with out selection pressure.

Correlation studies of screening at seed germination, seedling and adult plant stage (without the application of selection pressure) showed the survival percentage at seed germination stage was correlated significantly and positively with the survival at seedling and adult plant stage. Correlation between screening at seedling and adult plant stage was possitive but not significant. Nine accessions (IIHR 118, 119, 121, 43, 51, 45, 40, 46 and 33) remained resistant or highly resistant to Fusarium wilt in all the 3 stages. Three accessions (IIHR 133, 35 and 114) which were susceptible at seed stage became resistant at seedling and adult plant stage. Two accessions (IIHR 50 and

Table 16 : Comparison of screening watermelon accessions with and without selection pressure at adult plant stage

Lines	Survival (%)		
	Seed stage	With selection pressure at seed and seedling stages	Without selection pressure at seed and seedling stages
IIHR 118	100	100.00	100.00
IIHR 119	80	100.00	91.67
IIHR 121	80	100.00	87.50
IIHR 43	70	74.99	83.30
IIHR 133	50	100.00	80.67
IIHR 51	85	100.00	80.00
IIHR 35	40	70.83	79.20
IIHR 50	65	90.00	77.50
IIHR 138	80	100.00	73.20
IIHR 45	60	83.33	70.00
IIHR 40	80	85.70	70.00
IIHR 46	70	100.00	67.50
IIHR 114	50	87.50	66.67
IIHR 33	80	87.50	63.35
Sugar baby	00	00.00	00.00

138) which were resistant at seed stage, became susceptible at seedling stage and again became resistant to Fusarium wilt at adult stage.If we protect these lines at seed germination stage or seedling stage by giving treatment with fungicides, we may get resistant plants at seedling and adult stages, however, it needs further confirmation (Table 17).

The selfed progenies of the 15 accessions showing resistant reaction at all the 3 stages of plant growth were again screened for their level of resistance to Fusarium wilt at seed and seedling stage for confirming the previous results. When the results of screening at seed germination stage during the previous generation was compared with those obtained in the next generation it was found that out of 11 accessions which showed resistant or highly resistant reaction in the previous generation, two accessions (IIHR 138 and 45) became susceptible to Fusarium wilt whereas two accessions (IIHR 114 and 133) which were grouped as susceptible, became resistant during the next generation. One accession (IIHR 118) remained highly resistant (100 % survival) after selfing, in both the generation.

The results of screening of the selfed progenies at seedling stage were compared with the results of previous generation (Table 18), it was observed that out of 12 accessions which showed resistant to highly resistant reaction at seedling stage in the previous generation,3 accessions (IIHR 43,45 and 35) became susceptible, while one accession (IIHR 50) which was

Table 17 : Comparision of results of screening watermelon accessions at seed germination,seedling and adult plant stages

Lines	Survival percentage of plants		
	Seed stage	Seedling stage	Adult plant stage
IIHR 118	100	100	100
IIHR 119	80	67.50	91.67
IIHR 121	80	54.50	87.50
IIHR 43	70	78.38	83.30
IIHR 133	50	54.50	80.67
IIHR 51	85	80.00	80.00
IIHR 50	65	34.00	77.50
IIHR 35	40	59.00	79.20
IIHR 138	80	50.00	73.20
IIHR 45	60	78.50	70.00
IIHR 40	80	62.50	70.00
IIHR 46	70	73.50	67.50
IIHR 114	50	70.50	66.67
IIHR 33	80	86.00	63.35

Table 18 : Comparision of results of screening at seed and seedling stage of selfed progenies with their previous generation

Lines	Survival (%)			
	Seed stage		Seedling stage	
	Previous generation	Progenies	Previous generation	Progenies
IIHR 118	100	100.00	100.00	100
IIHR 51	85	70.00	80.00	75
IIHR 119	80	85.00	67.00	80
IIHR 40	80	79.15	62.50	80
IIHR 121	80	60.00	54.50	50
IIHR 33	80	70.00	86.00	60
IIHR 138	80	25.00	50.00	30
IIHR 43	70	70.00	78.38	50
IIHR 46	70	80.00	73.50	00
IIHR 50	65	55.50	34.00	65
IIHR 45	60	50.00	78.00	40
IIHR 114	50	60.00	70.00	60
IIHR 133	50	60.00	54.05	70
IIHR 35	40	45.00	59.00	40
IIHR 20	40	40.00	30.00	40

susceptible, became resistant upon selfing. The susceptible accession IIHR 20 remained susceptible and IIHR 118 remained highly resistant upon selfing.

Five accessions (IIHR 118, 51, 119, 40 and 33) remained resistant or highly resistant to Fusarium wilt in both the generations. Two accessions (IIHR 121 and 43) which were resistant to highly resistant in the previous generation became susceptible at seedling stage in the next generation. Two accessions (IIHR 114 and 139) which were susceptible at seed stage in the previous generation, became resistant after selfing. One accession (IIHR 50) which was susceptible at seedling stage in the previous generation became resistant upon selfing. This indicates that these lines are still segregating for their reaction to Fusarium wilt.

In the confirmatory test at seed germination stage 73.33 per cent of the progenies (11 out of 15 accessions) showed resistant or highly resistant reaction and 26.67 per cent of the progenies showed susceptible to highly susceptible reaction to Fusarium wilt.

During screening selfed progenies at seedling level 9 progenies out of 15 (60 %) showed resistant to highly resistant reaction and 40 per cent of the progenies showed susceptible to highly susceptible reaction towards Fusarium wilt.

Thus with one cycle of selection for resistance to

Fusarium wilt at seed germination, seedling and adult plant stage, the per cent of resistant progenies increased from 19.64 (prior to selection) to 73.33 in the selfed progenies at seed germination stage viz., 311.85 per cent increase in the number of resistant progenies over the previous cycle of selection. This clearly indicated that the original accessions screened for resistance to Fusarium wilt during first generation of selections, were segregating for resistance to Fusarium wilt and selection pressure helped in increasing the frequency of resistant gene(s) in the next generation. Four resistant or highly resistant progenies, IIHR 118, 119, 40 and 33, gave consistant results under all the methods of screening, in both the generations The consistant results of the above progenies could be due to their genetic make up i.e., single dominant gene for resistance to Fusarium wilt as reported by Netzer (1982).

Seven accessions (IIHR 43, 121, 45, 35, 20, 138 and 46) showed susceptible to highly susceptible reaction in the second generation. It could be due to the heterozygus condition of the first generation which on selfing became homzygus for susceptibility.

The standard watermelon varieties like sugar Baby and Arka Manik were highly susceptible to Fusarium wilt.

SUMMARY

VI SUMMARY

Experiments were conducted to standardise screening techniques as well as locating the sources of resistance to Fusarium wilt in watermelon during 1992-93 at IIHR, Bangalaore. The following is the list of summary of results.

1. Soaking the seeds in the fungal inoculum of concentration 1×10^6 spores/ml for 48 hour and placing the seeds on the fungal mycelium grown on germination paper inside the petriplates and incubating for 5 days at 27^oC was found to be the best treatment for screening watermelon accessions for resistance to Fusarium wilt at seed germination stage.

2. Growing medium composed of organic mixture and sorghum grains in the proportion of 5:1 was found to be the most suitable medium for the simultaneous growth of fungus and watermelon seedlings.

3. Cutting the roots to a distance of 2mm from the tip and dipping in the fungal inoculum of concentration 1×10^6 spores/ml for 48 hours, and transplanting the seedlings to the polythene bags in which fungus was grown on a standardized medium composed of organic mixture and sorghum for 7 days was found to be the best screening technique at seedling stage.

4. Incorporation of the fungus grown on a standardized medium composed of organic mixture and sorghum grains in to root zone at the adult stage (one month after sowing) was found to be the best

method for screening watermelons accessions at adult stage.

5. Screening at seed germination stage was the quickest screening technique which gave the results in 5 days when compared to 20 days and 55-60 days while screening at seedling and adult plant stage respectively.

6. A total of 56 accessions were screened against Fusarium wilt using seed, seedling and adult plant inoculation methods. Under Screening at seed stage, seven accessions (IIHR 118, 119, 121, 51, 40, 33 and 138) were found highly resistant (80-100 % survival) and four accessions (IIHR 43, 46, 50 and 45) were found resistant to Fusarium wilt.

7. Under screening at seedling stage without selection pressure four accessions (IIHR 118, 33, 51, 45 and 43) were found highly resistant and seven accessions (IIHR 46, 114, 119, 40, 35, 121 and 133) were found resistant to Fusarium wilt.

8. Under screening without selection pressure at adult plant stage 8 accessions (IIHR 118, 119, 121, 43, 133, 51, 35 and 50) were found highly resistant and 6 accessions (IIHR 138, 45, 40, 46, 114 and 33) were resistant to Fusarium wilt. One accession (IIHR 118) showed a 100 per cent survival in all the 3 stages. Nine accessions (IIHR 118, 119, 121, 43, 51, 45, 40, 46 and 33) remained resistant or highly resistant to Fusarium wilt in all the 3 stages of screening. There was a significant and positive correlation between survival percentages of screening at seed

germination stage with screening at seedling and adult plant stages.

9. Under screening with selection pressure 7 accessions (IIHR 118, 119, 51, 121, 40, 33 and 138) remained highly resistant and 4 accessions (IIHR 43, 46, 40 and 45) remained resistant to Fusarium wilt at all the 3 stages of development.

10. Fifteen selfed progenies were subjected to seed germination and seedling inoculation methods of screening. Five accessions (IIHR 118, 51, 119 40 and 33) remained resistant or highly resistant at both seed germination and seedling stages even after selfing.

11. One accession (IIHR 118) showed 100 per cent survival in the original population as well as in the next generation obtained on selfing, both at seed and seedling stages.

12. Commercial varieties like Arka Manik and Sugar Baby were found to be highly susceptible to Fusarium wilt.

Future lines of work

1. Identification of races of Fusarium oxysporum f.sp. niveum
2. Confirmation of resistance to Fusarium wilt in the resistant and highly resistant lines and in their progenies using larger populations.

3. Determination of genetics of resistance to Fusarium wilt in watermelon.

4. Determination of mechanism of resistance to Fusarium wilt in watermelon.

5. Transfer of resistant gene(s) to commercial watermelon varieties.

6. Biological control of Fusarium wilt using biological agent like *Trichoderma harzianum*.

REFERENCES

VII REFERENCES

*Akhavizadegan, M.J., 1983, Occurence of Fusarium wilt of water watermelon in Guilan province of Iran. ***Entomologiae,*** et ***Phytopathologic. Appliques,*** 50 (1/2) : 67-73.

Armstrong, G.M. and Armstrong, J.K., 1978, Formae specials and races of ***Fusarium oxysporum*** causing wilt of the cucurbitaceae. ***Phytopathology,*** 68 (1) : 19-28.

Barna, B., 1980, Reliability of yield of watermelon hybrids resistant to disease. ***Rev. Plant Pathol.,*** 59 (1) : 526.

Barnes, G.L., 1972, Differential pathogensity of ***Fusarium oxysporum f.sp. niveum*** to certain wilt resistant watermelon cultivars. ***Plant, Dis. reptr.,*** 56 (2) : 1022-1026.

*Bastos Cruz, B.P., Silveiria, A.P. and Mendonca, N.T., 1968, Determination of the resistance of some watermelon varieties to Fusarium wilt [***Fusarium oxysporum (schlett)] f. niveum [E.F.Sm]*** Synder and Hansen], ***Arq. Inst. Biol. Sci. Paulo.,*** 35 (2) : 67-74.

*Beider, A.M., 1977, Rapid method of evaluating watermelon varieties for rersistance to Fusarium wilt. ***Referativnyi. Zhurnal.,*** 5 : 65 : 530.

*Belik, V.F., Vdouina, I.A. and Nekrasov, N. YA., 1974, Evaluating resistance to ***Fusarium oxysporum*** in watermelon by water balence. ***Referativnyi Zhurnal,*** 1 : 55 : 661.

Bhide, V.P., Hegde, R.K. and Sukapure, R.S., 1955, Fusarium wilt of watermelon and peas in Bombay state. ***Curr. Sci.,*** **24** : 310.

Booth, C., 1979, ***Fusarium*** : Laboratory guide to the identification of major species. Academic Press, New York, 13 pp.

Crall, J.M. and Elmstorm, G.W., 1981, Sugarlee, an early, high quality, disease-resistant watermelon variety for Florida commercial growers. *Agric. Expt. Stn. Florida. Uni.,* 5 : 277-278.

*Cruz, B.P.B., Silveira, A.P. and Silveira, J.P., 1967, Occurence of watermelon wilt in the state of Sao Paulo. *Biologico,* 33 (2) : 40-41.

*Cruz, B.P.B., Silveira, A.P.D.A. and Mendonca, N.T. DE., 1968, Determination of the resistance of some watermelon varieties to Fusarium wilt. *Arq. Inst. Biol. Sci. Paulo.,* 35 (2) : 67-76.

*De Candolle, A., 1882, Origin of cultivated plants. New York.

Elmstorm, G.W. and Crall, J.M., 1979, "Dixilee" watermelon : disease resistance, quality and yield evaluation. *Hort. Sci.,* **14** (2) : 122.

Elmstrom, G.M. and Hopkins, D.L., 1981, Resistance of watermelon cultivars to Fusarium wilt. *Plant, Dis.,* 65 (10) : 825-827.

*Erwin, F., Smith, 1899, Wilt disease of cotton, watermelon and cowpea. *U.S. Dept. Agr. Div. Veg. Phys. and Path. Bull.,* 17.

*Ester, A. and Gerlagh, M., 1980, *Fusarium oxysporum f.sp. melonis* a risk factor in melon growing. *Institut. Voor. Plantenziektenkundig onderzoek.,* 34 (5) : 134-136.

*Ester,A. and Gerlagh, M., 1981, *Fusarium oxysporum f.sp. melonis* a risk factor in melon culture. *Zaadbelangen.,* 34 (5) : 134-136.

*Feher, T., 1986, Evaluation of some new Keskmet F_1 watermelons with special refference to Fusarium resistance. *Zoldlegtermesztesi, Kutato, Insterzet, Bulleteinje.,* **19** : 35-39.

Gill, H.S. and Tomer, B.S., 1991, Vegetable statistics at a glance. *P.D.V.R., Tech. Bull.*, **4** : 16

Hegde, R.K. and Bhide, V.P.,1958, Watermelon wilt in Bombay state caused by *Fusarium oxysporum f. niveum* (E.F.Smith) Snyder and Hansen. *Indian Phytopath.,* 11 : 49-52.

Henderson, W.R., Jenkins, S.F. and Rawlings, J.O., 1970, The inheritance of Fusarium wilt resistance in watermelon *Citrullus lanatus (Thunb.). Hort. Sci.,* 95 (3) : 276-282.

Herklots, 1972, Vegetables in South east asia, *London George Allen and Unwin Ltd.*

Hopkins, D.L. and Elmstorm, G.W., 1983, Fusarium wilt in watermelon cultivars grown in a 4 year monoculture. *Plant Dis.,* 68 (2) : 129-131.

*Huang, J.W., Sun, S.K. and Lo, C.T., 1982, Screening for Fusarium wilt resistance in watermelon seedlings with toxic metabolites extracted from *Fusarium oxysporum f.sp. niveum. Plant. Prodn. Bull. Taiwan.,* 61 (11) : 6652.

Jhamaria, S.L. and Patel, P.N., 1971, Fusarium wilt of watermelon in Rajasthan. *Sci. and Cult.,* 37 : 45-46.

*Jiang, M.X., 1981, Selection and evaluation of the cucumber hybrids, Ninghuang 1 and Ninghuang 2. *Agric. Sci. and Tech.,* 5 : 10-11.

Kannaiyan, S. and Purushothaman, D., 1973, Distribution of cucurbitacin in Muskmelon fruits in relation to Fusarium wilt resistance. *Madras Agric. J.,* 60 (7) : 614-615.

Kesavan, P. and Prasad, N.N., 1979, Correlation between crude cucurbitacin content in certain muskmelon varieties and Fusarium wilt incidence. *Indian. J. Expt. Biol.,* 12 (5) : 476-477.

*Khodzhayan, E. and Babayan, A.A., 1963, On the specialization of forms of *Fusarium oxysporum*, the causal agent of wilt in cucurbit culture. *Biol. Sci.,* 16 (8) : 65-72.

*Kim, M.S., Ban, C.D., Kang, K.Y. and Kim, H.T., 1984, New hybrid netted melon cultivar Poogmi for plastic film house cltivation. *Res. Rep.* office at the Rural Development. *S.Korea, Horticulture.,* 26 (2) : 73-75.

Malepsy, S. and El-Kazzaz., 1990, In vitro culture of *Cucumis sativa* XI. Selection of resistance to *Fusarium oxysporum. Acta. Hort.,* 280 : 455-458.

Martyn, R.D., 1986, A new race of the Fusarium. Watermelon wilt fungus in the United States. *Hort. Sci.,* 21 (4) : 941.

Martyn, R.D. and Burton, B.D., 1989, An initial survey of *Fusarium oxysporum f.sp. niveum* races in the United States. *Hort. Sci.,* 24 : 696-698.

Martyn, R.D. and McLaughin, R., 1982, Resistance and susceptibility of *cucurbita* spp. to *Fusarium oxysporum f.sp. niveum. Phytopathology.,* 72 (3) : 359.

Martyn, R.D. and McLaughlin, R.J., 1983, Effect of inoculum concentration on the apperent resistance of watermelon to *Fusarium oxysporum f.sp. niveum. Plant. Dis.,* 67 (3) : 493-495.

*Mas, P., 1973, Research on Fusarium disease XI. Penentration of *Fusarium oxysporum f.sp. niveum* into resistant and susceptible melon seedlings using two inoculation techniques. *Ann. phytopathol.,* 5 (2) : 213-218.

*McArdle, R.N. and Kendall, S.A., 1983, Peat pellet inculation : a potential rapid screening method for *Fusarium* resistance in *cucumis melo. Cucurbit. Genetic. Co-op.,* 6 : 43-44.

*McMillan, R.T., 1986, Cross pathogencity studies with isolates of *Fusarium oxysporum* from either cucumber or watermelon pathogenic to both crop species. *Ann. app. Biol.,* 101-105.

*Megnegneau, B. and Branchard, M., 1988, Toxicity of fusaric acid observed on callus cultures of various *cucumis melo* genotypes. *Plant. Physiol. Biochem. France.* 26 (5) : 585-588.

*Michail, S.H., Ibrahim, I.A., Abdel Rehim, M.A. and Fadel, F.H., 1971, Damping off of cucurbitacious plants in U.A.R. III. *Fusarium Semitectum,* Bark. et Rev., a damping causal organisum of watermelon. *Phytopathol. Meditteranian.,* 10 (1) : 46-49.

Mituskovic, M. and Vucinic, Z., 1977, Fusarium wilt of watermelon in Montenegro and the possibility of its control. *Plant. Pathol.,* 58 : 5.

Mohammed, M.A., Hassan, A.A., Oksh, I.I. and Hilal, R., 1981, Nature of resistance to Fusarium wilt in watermelon. *Egyptian. J. Hort.,* 8 (1) : 1-12.

Mondal, S.N. and Rashid, M.A., 1990, Varietal resistance of watermelon to Fusarium wilt. *Bangladesh J. Agric. Res.,* 15 (1) : 75-76.

*Nepa, G.A.W., Rhodes, B.B. and Witcher, W., 1985, Interaction of commercial watermelon cultivar's with regional isolates of *Fusarium oxysporum f.sp. niveum. Cucurbit Gene. Co-op.,* 8 : 62-64.

Netzer, D., 1982, Resistance to *Fusarium oxysporum* : inheritance in watermelon and cotton and mechanism in cotton, *Phytoparasitica.,* 10 (2) : 131.

Netzer, D. and Martyn, R.D., 1989, P.I. 296341, a source of resistance in watermelon to race 2 of *Fusarium oxysporum f.sp. niveum. Plant. Dis.,* 73 (6) : 518.

Netzer, D., Niego, S. and Gulum, E., 1977, A dominant gene conferring resistance to Fusarium wilt in cucumber. *Phytopathology.,* 67 (4) : 525-527.

*Netzer, D., Kritzman, G. and Ghet, J., 1979, B-(1,3)-glucanase activity and quantity of fungus in relation to Fusarium wilt in resistant and susceptible near isogenic lines of muskmelon. ***Physiol. Plant pathol.,*** **14** (1) : 47-55.

Netzer, D. and Weintall, C., 1980, Inheritance of resistance in watermelon to race 1 of *Fusarium oxysporum f.sp. niveum. Plant. Dis.,* **64** (9) : 853-854.

*Nikiforova, N.A., 1968, Vaccination as a method of increasing the Fusarium resistance of watermelon. *Vest Sel-Khoz Nauki Mosk.,* 13 (2) : 40-43.

Norton, J.D., Cosper, R.D., Smith, D.A. and Rymal, K.S., 1985, Au-Jubilant and Au-Producer, quality, disease-resistant watermelon varieties for the South. *Circular. Agric. Expt. Stat. Aubum. Uni.,* **280** : 11.

Ortan, W.A., 1907, A study of disease resistance in watermelon. *Science* 25 : 258.

Palmer, M.J. and Williams, P.H., 1981, A seedling evaluation method for Fusarium wilt of cucumber initiated by *Fusarium oxysporum f.sp. cucumerinum. Phytopathology.,* **71** (2) : 247.

Paulus, A.O., Harvey, O.A., Nelson, J. and Shibuya, F., 1976, Fusarium resistant watermelon cultivars. *California. Agric.,* 30 (9) : 5-6.

*Pelcz, J., 1984, Studies on methods of testing and assessing resistance in cucumber to *Fusarium oxysporum* Schletcht. emend. Sny and Hans f.sp. *Cucumerinum* owen and *Fusarium solani.* (Mart) Sacc. f.sp. *cucurbitae* Sny and Hans. *Archiv. fur. Zuchtungs forshung.,* **14** (3) : 209-214.

Radhakrishna, P. and Sen, B., 1985, Efficacy of different methods of inoculation of ***Fusarium oxysporum and Fusarium Solani.*** for inducing wilt in musk melon. ***Indian Phytopath.,*** 38 (1) : 70-73.

*Risser, G., 1973, Study of the inheritance of resistance to race 1 and 2 of ***Fusarium oxysporum f.sp. melonis*** in melons. ***(Cucumis melo). Annales de i Araelioration des plantes.*** 23 (3) : 259-263.

*Simon, A.M. and Galaev, M.K.H., 1985, A modification of the microhydrophonic method of determining disease resistance in vegetable crops as applied to watermelon. ***Shornik. Nauchnykh Trudov Po. Priklandori Botanike Genetike i selektsii.,*** 92 : 118-119.

Singh, R.S., 1982, Plant pathogens. The fungi. ***Oxford and I.B.H. Publishing Co., New Delhi,*** 350-353.

*Sugahara, Y., 1974, Melon breeding I. Methods of assessing resistance to Fusarium wilt and sources of disease resistance in cucurbitaceae. ***Bul. Veg. Ornament. Crop. Res. Stat.,*** 1 : 15-17.

Sundararaj, N., Nagaraju, S., Venkataramu, M.N. and Jagannath, M.K., 1972, Design and analysis of field experiments, UAS, Hebbal, Bangalore. 141-167.

*Tekanovich, G.A. and Fursa, T.B., 1975, Evaluation of the watermelon collection for resistance to Fusarium wilt. ***Kartofel's ovoshchi.,*** 11 : 40.

*Timchenko, V.I. and Beider, O.M., 1977, Effect of culture liquid of ***Fusarium oxysporum f.sp. niveum*** on the resistance of watermelon to Fusarium wilt. ***Referativnyi Zhurnal.,*** 8 :55:1309.

*Timchenkov, V.I. and Beider, A.M.,1979 (a),laboratory method for the rapid evaluation of watermelon varieties and hybrids for resistance to Fusarium. ***Referativnyi Zhurnal.,*** 10: 55:453.

*Timchenkov, V.I. and Beider, A.M., 1979 (b), Anatomical and morphological features of watermrelon plants resistant to Fusarium wilt. ***Referativnyi Zhurnal.,*** 11 :55:327.

Vdovina, T.,A. and Belik, V.F., 1974, Physiological evaluation of resistance to Fusarium wilt in melon crops. ***Plant. Breed. Abstr.,*** 8611.

*Wang, M. and Zhang, X., 1988, Studies on watermelon germplasm sources of resistance to Fusarium wilt disease at the seedling stage. ***Cucurbit. gen. co-op.,*** 11 : 68.

*Yu, S.Q. and Wang, S.Z., 1990, Study on appraisal method for assessing resistance to fusarial wilt disease in watermelon. ***Scientia. Agri. Sinia.,*** 23 (1) : 31-36.

*Zhang, Y.W., Quin, X.L. and Gu, Q.S., 1991, Studies of methods for identifying resistance in watermelon to Fusarium wilt. ***J. Fruit. Sci.,*** 8 (4) : 219-224.

Zink, F.W., Gubler, W.D. and Grogan, R.G., 1980, Inheritance of Fusarium resistance in muskmelon. ***Hort. Sci.,*** 15 (3 II) ; 418.

Zink, F.W., Gulber, W.D. and Grogan, R.G., 1982, Genetics of Fusarium wilt resistance in muskmelon. ***Hort. Sci.,*** 18 (4 II) : 602.

Zink, F.W. and Gubler, W.D., 1985, Inheritance of resistance in muskmelon to Fusarium wilt. ***J. American. Soc. Hort. Sci.,*** 110 (5) : 600-604.

Zink, F.W. and Gubler, W.D., 1986 a, U.C.PMR 45, Fusarium wilt resistant, muskmelon breeding line. ***Hort. Sci.,*** 21 (2) : 333.

Zink, F.W. and Gubler, W.D., 1986 b, Inheritance of resistance to race 0 and 2 of ***Fusarium oxysporum f.sp. melonis*** in a gynocious muskmelon. ***Plant. Dis.,*** 70 : 676-678.

Zink, F.W. and Thomas, L.E., 1990, Genetics of resitance to ***Fusarium oxysporum f.sp. melonis*** race o, 1 and 2 in muskmelon line MR1. ***Phytopathology.,*** 80 (11) : 1230-1232.

*** Originals not seen**