A Screening Technique for Resistance to Vascular Wilt in Lentil

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Abstract

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Vascular wilt caused by *Fusarium oxysporum* f.sp. *lentis* is the major disease on lentil *(Lens culinaris)* in Syria. Although extensive screening has been done in the field at ICARDA, it was necessarily opportunistic because of the uneveness of wilt distribution. A simple, rapid and repeatable technique has been developed in the plastic house to screen lentil germplasm at the seedling stage for resistance to wilt. The technique involved planting of one row of each of the test lines with a susceptible check at every 5th row in metal trays containing field soil and inoculation of 14-day old plants with a liquid culture of *F. oxysporum* isolated from the stems of wilted plants. Final disease incidence was recorded eight weeks after sowing. A total of 162 lines were screened using this technique and 29 suggested presence of resistance as no disease developed. The repeatability of the technique was high with a correlation of r = 0.86 ($P < 0.01$) between repeated sowings of 25 lines. Eighteen of the lines were grown in the field where their reaction was same as in the plastic house.

Key words: wilt, lentil, Syria.

Introduction

Vascular wilt caused by *Fusarium oxysporum* f.sp. *lentis.* (Vasudeva and Srinivasan) Gordon is the major disease of lentil (*Lens culinaris* Medic.) in Syria (2, 3). Although an overall estimate of 12% crop loss from wilt has been made for North-West Syria, the distribution of the disease within fields is usually patchy (2). This uneven distribution makes screening for disease reaction in the field difficult. Opportunistic screening of a total of 933 breeding lines and germplasm accessions has been undertaken in the field at ICARDA since 1986 (4,5) revealing a wide range in reaction to wilt.

Late sowing in February compared with normal sowing time (December) increased disease incidence from 0.6 to 9.3%. The range in response amongst test material was also greater in late (0-100% wilted palnts) compared to earlier sowing dates (0-10%) (5). Although late sowing may improve field screening through an increase in wilt incidence and the range of its incidence, the uneven distribution of the disease remains a problem. Therefore, this study was undertaken to develop a reliable and simple method to create epiphytotics with a uniform disease pressure to test genetic material for resistance to fusarium wilt of lentil.

Materials and Methods

Screening for fusarium wilt was done in metal trays (60 × 45 × 10cm) filled with 25kg of natural field soil to a depth of 5cm placed in a plastic house maintained at 20 ± 4°C at Tel Hadya, Syria. In every tray one row of 15 seeds of each of six lentil genotypes, including a susceptible check (ILL 4605), was sown. The genotypes were arranged in a randomized block design with two replications. Trays were watered weekly.

Two week-old seedlings were inoculated with fourteen day-old culture of *Fusarium oxysporum* f.sp *lentis* previously isolated from the stems of wilted lentil plants (2). Inoculum was prepared by adding 10 discs (5 mm diameter) covered with fungal growth, obtained from the margin of an actively growing colony to 250 ml of lentil-seed extract dextrose medium (6) in 500 ml Erlenmeyer flasks incubated at 20 ± 1°C.

After 14 days of incubation, 250 ml of sterilized water was added to each flask, blended in a waering liquidizer for one minute and distributed evenly at the rate of one flask per tray into furrows ca3 cm deep prepared between rows. Following inoculation the furrows were closed.

The seedlings were scored for disease reaction after eight weeks as follows: 1=healthy, 2=with some wilting but alive, 3=dead. Two values for each row were derived from the data: firstly, the percentage of dead wilted plants and secondly, a disease index in percent (7) calculated as:

$$\frac{\text{(Numerical ratings)} \times 100}{\text{Number of seedlings} \times \text{maximum disease rating}}$$

To test the repeatability of the technique, the screening of 25 elite lentil lines varying from resistant to susceptible from the ICARDA collection was repeated twice. An additional 137 elite lines were screened in three consecutive sowings, giving an overall total of 162 lines tested.

In order to ensure a valid comparison between sowings and trays, a population approach to classify disease reaction was taken using a 1 - 9 scale based on the deviation from the overall mean for disease index of the susceptible check as follows:

Rating

1. (Highly resistant) disease index of 33.3%.

3 (Resistant) more than 33.3% disease index and a value

less than three standard deviations below the overall mean of the susceptible check.

5 (Moderately susceptible) between 2 and 3 standard deviations below the overall mean of the susceptible check.

7 (Susceptible) within 2 standard deviations of the overall mean of the susceptible check.

9 (Highly susceptible) equally or more susceptible than the overall mean of the susceptible check.

Field data on disease reaction were collected on 18 lines, also grown in the plastic house. These lines were sown in the crossing block at Tel Hadya farm, N. Syria in December 1987 in individual rows which were 3m long and 60 cm apart. As parents in the crossing block the number of replicates per line varied from 5 - 25 with a mean of 11. Wilt incidence was assessed on May 9, 1988 during the pod filling stage as the percentage of wilted plants per row. The disease reaction (% wilted plants) of a line in the field was based on a 1 - 9 scale as follows:

Rating	
1 (Highly resistant)	no wilted plants
3 (Resistant)	1 - 33% wilted plants
5 (Moderately susceptible)	34 - 66% wilted plants
7 (Susceptible)	66 - 99% wilted plants
9 (Highly susceptible)	all plants wilted

Since we presume that low scores represent disease escape, only the highest score of a line was used.

Results

Repeatability of seedling wilt reaction in the plastic house. The 25 lines tested repeatedly ranged from resistant, to highly susceptible. There was good agreement between the results of consecutive sowings with a correlation of r= 0.86 ($P < 0.01$) between the respective disease indices (Figure 1). This shows that the observed resistance is genotype determined. The association between disease reaction as measured by % wilted plants in consecutive sowings was lower at r = 0.71 ($P < 0.01$) than the disease index.

Association between field and plastic house estimates of disease reaction. The uneven distribution of the disease across the experimental field is illustrated by the varied disease reaction of different individual rows of one of the lines -- ILL 4354. The range for this line was from 0-90% wilted plants per row with an overall mean of 32.4% wilted plants over 25 rows.

A total of 18 lines were screened in both the field and plastic house and the range for the highest score amongst the lines was 0-100% wilted plants in the field. The correlations between field and plastic house estimates of disease reaction are shown in Table 1. There were higher correlations between the field and plastic house results with disease index data than % wilted plants data, although all the correlations were significant. The highest correlation was between the wilted plants score in the field and disease index score in the plastic house with r=0.76 ($P<0.01$). This association is illustrated in Figure 2.

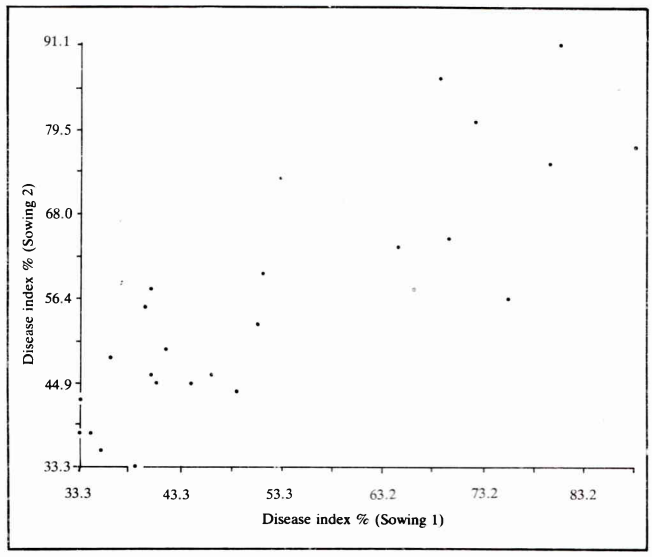

Figure 1. Disease index (%) of 25 lines of lentil in repeated sowings in the plastic house.

Table 1. Correlation coefficients (r) between field and plastic house estimates of disease reaction of lentil to fusarium wilt.

Field	Plastic house			
	% wilted plants	Wilted plants (1-9 scale)	Disease index	Disease index (1-9 scale)
% wilted plants in field	0.61**	0.52*	0.66**	0.74**
Wilted plants (1-9 scale) in field	0.63**	0.58*	0.67**	0.76**

* $P < 0.05 > 0.01$
**$P<0.01$

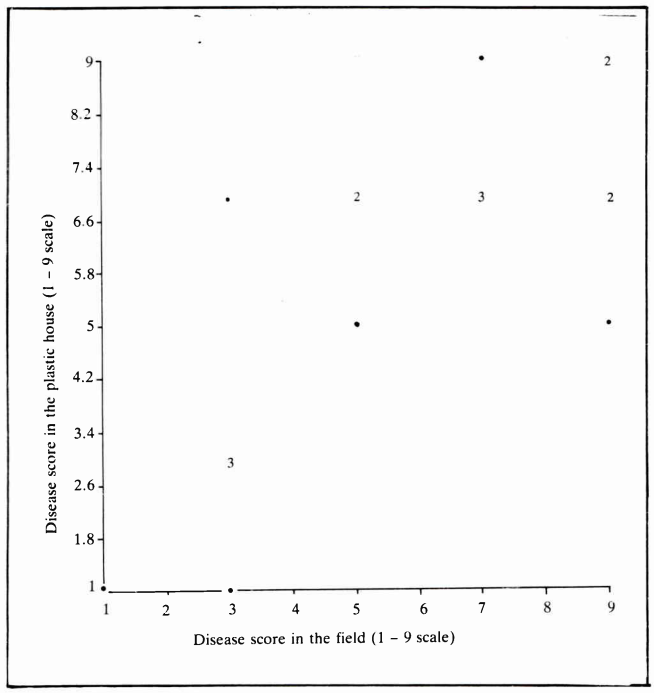

Figure 2. Disease rating on 1 - 9 scale of 18 lines of lentil in the field and the plastic house.

Table 2. Distribution of 162 lentil lines in reaction to fusarium wilt.

Disease reaction	Highly resistant	Resistant	Moderately susceptible	Susceptible	Highly susceptible
Score	1	3	5	7	9
Number of lines	29	36	35	47	15
% of total lines	18	22	22	29	9

Overall genetic variation in disease reaction in the plastic house. A total of 162 lines were screened in the plastic house. Twenty nine of these were apparently highly resistant showing no wilt symptoms, which represents 17.9% of the total population screened. The distribution of 162 lentil lines in reaction to wilt is shown in Table 2 with a list of the reaction of individual lines given in Table 3.

Table 3. Disease rating of 162 lentil lines (ILL accession numbers) on 1 - 9 scale on basis of fusarium wilt index in plastic house.

1 Highly resistant	3 Resistant		5 Moderately susceptible		7 Susceptible		9 Highly susceptible
5582	2126	6244	2022	6434	468	6201	4711
5588	2130	6245	3601	6440	4354	6202	5544
5722	2582	6253	4349	6441	5590	6206	5604
5751	3607	6254	4668	6448	5627	6211	5728
5845	3614	6260	5700	6457	5668	6213	5860
5996	4403	6437	5714	6473	5671	6236	5863
6050	5715	6442	5752		5729	6238	5871
6207	5748		5825		5754	6241	5999
6215	5760		5830		5758	6246	6002
6216	5766		5989		5814	6248	6003
6217	5775		5991		5815	6255	6035
6220	5828		5999		5816	6256	6208
6221	5836		6042		5840	6257	6209
6222	5842		6148		5864	6262	6210
6223	5854		6192		6004	6263	6247
6226	5869		6194		6015	6265	
6227	5876		6195		6016	6266	
6228	5988		6198		6017	6444	
6229	5994		6203		6018	6452	
6233	6010		6204		6019		
6234	6025		6205		6021		
6239	6214		6235		6027		
6243	6218		6237		6036		
6249	6219		6259		6049		
6250	6224		6261		6191		
6409	6225		6264		6193		
6430	6230		6431		6196		
6435	6231		6432		6197		
6474	6232		6433				

Discussion

Screening methods for disease resistance should be simple, rapid, reliable, and, preferably, non-destructive, so the assayed resistant plants can give seed. The method developed herein is both simple and rapid-taking eight weeks. This allows several cycles of screening per crop season. This method showed a high repeatability by the association (r=0.86) between estimates of disease reaction from repeated screenings of 25 lines. Additionally, results from the field agreed with those obtained from the plastic house. Since the disease distribution in the field was not uniform, the field data may overestimate the resistance through disease escape. However, lines showing susceptibility in the field are truly susceptible. All lines rated as 5 or more in the field were also rated as 5 or more in the plastic house.

The field data were collected during the pod-filling stage of reproductive growth, whereas the plastic-house screening was conducted on eight-week old seedlings. The optimum temperature for growth of *F. oxysporum* f.sp. *lentis* has been established as 22°C (6). The plastic house was maintained at

around 20°C to enhance infection. The late manifestation of disease symptoms in the field which occurs during crop reproductive growth of the crop may be because temperature affects fungal growth (6).

In the plant breeding of autogamous crops, a non-destructive screening techniques is preferable to a destructive one since it allows seed collection from resistant plants during the screening of segregating material. In this technique 90 seeds were sown into 25kg of soil at the rate of 333 seeds m^{-2}. After the completion of screening on eight-week old seedlings, it should be possible to allow the remaining resistant plants to grow to test their reaction to the disease at the adult stage and eventually to produce seed following the application of appropriate inputs (fertilizer etc.) to the trays.

The data on lentil wilt reaction from the field were opportunistic since we had no fore-knowledge of wilt distribution within the field. Another approach to screening of root diseases is to develop and use a «sick» plot with a uniform distribution of pathogen within the appointed area. One risk of the «sick» plot is possible pathogen spread to other experimental areas or fields (1). The use of the plastic house technique and a «sick» plot are complementary with the former allowing the rapid screening out of susceptible lines leaving a few, more resistant lines for field testing over a complete growing season.

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الملخص

بياعة، بسام وويلي أرسكين. 1990. تقنية لتقويم مقاومة سلالات العدس لمرض ذبول العدس. مجلة وقاية النبات العربية 8(1): 30 - 33.

يعتبر مرض ذبول العدس الذي يحدثه الفطر *Fusarium oxysporum* f.sp. *lentis* المرض الرئيس الذي يصيب العدس في سورية. ورغم إجراء تقويمات حقلية شاملة لسلالات العدس في محطة المركز الدولي للبحوث الزراعية في المناطق الجافة (ايكاردا)، إلا أنها كانت تخضع لعامل الصدفة نظراً للتوزع غير المتجانس للقاح الفطري في التربة. وعليه فقد تمّ تطوير تقنية بسيطة، وسريعة، وقابلة للتكرار لتقويم مقاومة أصول العدس الوراثية وهي في مرحلة البادرة لمرض الذبول في ظروف الدفيئة البلاستيكية. وتتضمن التقنية زراعة السلالات المختبرة في صوان معدنية مملوءة بتربة حقلية، بواقع صف واحد من بذور الشاهد عالي الحساسية، وصف لكل من السلالات المراد اختبارها، والقاح البادرات بعمر 14 يوماً بمزرعة سائلة من الفطر *F. oxysporum* الذي سبق عزله من سوق نباتات مصابة بالذبول، وتسجيل وقوع الاصابة بعد ثمانية أسابيع من الزراعة. هذا وقد تمّ تقويم 162 سلالة باستخدام هذه التقنية، وتبيّن وجود 29 سلالة مبشرة لم تبد أعراضاً مرضية. وكان معامل الارتباط عند تكرار تقويم 25 سلالة بمواعيد مختلفة عالياً (r = 0.86 عند مستوى احتمال 1%) الأمر الذي يشير إلى صلاحية هذه التقنية. كما كان تفاعل ثمان عشرة سلالة من العدس في الحقل مشابه للتفاعل الذي أعطته هذه السلالات في ظروف الدفيئة.

كلمات مفتاحية: ذبول، عدس، سوريا.

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