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U.S. - ISRAEL COOPERATIVE DEVELOPMENT RESEARCH PROGRAM (CDR)

SCREENING, TRANSFER AND INCORPORATION OF ALIEN GENETIC MATERIAL INTO WHEAT GENOMES FOR RESISTANCE TO THE "TAKE-ALL" DISEASE

Project No. C5-039

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INTRODUCTION

In the previous report, the reaction of accessions of wild emmer wheat (Triticum dicoccoides) to Gaeumannomyces graminis var. tritici (GGT) were presented. Several accessions showed some degree of resistance, when assessed by using the Conical Tube method. At this stage of the selection work it was decided to concentrate on establishing the methodology of assessment, thereby enhancing the reliability of the results, as well as testing the consistency of reaction of accessions assessed by the Conical Tube method. The work reported here contains, in addition to further screening of genetic sources, experiments to test the effects of the inoculum type and dose, location of inoculation, and the environment where host-pathogen interaction takes place, on the infection. Likewise, assessment criteria were evaluated. All these studies were aimed toward develping a fast and reliable procedure for screening genetic sources resistant to take-all.

I. SCREENING TRITICUM DICOCCOIDES FOR RESISTANCE TO GGT

Methodology:

1a. Conical Tube Assay

Plastic 15ml test tubes (115 x 15mm internal height x diam., with drainage hole cut at the tip of the conical bottom) were filled with unsterilized vermiculite up to 4.5cm below the rim. PDA disks 13mm diam with 1-2wk-old GGT mycelium, or other types of inoculum, were placed on the vermiculite and covered by 2.5cm of vermiculite. Cereal seeds, one per test tube, pregerminated for 48h, were placed in the tubes and covered by an additional 1cm layer of vermiculite. Tubes were irrigated to field capacity with tap water. A small cottonwool plug at the drainage hole prevented loss of vermiculite. Tubes were held vertical in plastic raks, in an airconditioned growth room, at 18 C, and were irrigated twice weekly. Disease assessment was carried out 21 days after planting: plants were taken out of tubes and their root system was washed free of vermiculite. Each seminal root was assessed for discoloration due to GGT, and given an infection score (0-5 or 0-4) as described below. Each plant was given an infection score which was the

average of its root scores. Infection scores of plant accessions are averages of five (usually 10) or more plants/assay.

1b. A slightly different test tube assay was used in Portugal: test tubes, 160 mm high and 30 mm in diam., were filled with wetted vermiculite and autoclaved for 30 min. Three PDA disks, 12 mm in diam. cut from the growing margins of GGT colonies were used to inoculate each tube. Three pregerminated wheat seeds were sown in each tube, one on each PDA disk, and were covered with a layer of wet, sterilized vermiculite. Incubation was at 19±2 C in a growth room with a 15 h light photoperiod and 75% RH. Pathogenicity of GGT isolates was assessed after 15 days: plants were removed from the tubes and their roots were washed free of vermiculite. The pathogenicity score was a compound one, taking into consideration the discoloration of seminal roots, the density of runner hyphae, and symptoms on the aerial parts of the plants. The test tube assays were compared with different other assays throughout this study.

2. Root Discoloration Infection Scores (0 - 5)

0= apparently healthy
1= discoloration of up to 10% of the root length
2= discoloration of 11-25% -"-
3= discoloration of 26-50% -"-
4= discoloration of 51-75% -"-
5= discoloration of 76-100% -"-

1a: Screening Israeli *Triticum dicoccoides* accessions

(a) GGT isolate: Galon '87.
(b) Inoculum types: PDA disks.
(c) Test plants: *T. dicoccoides* 41 accessions; Standards:wheat cv. "Barkai" (susceptible); barley "DRO 32" (intermediate); rye "Wesser" C.I.33 (resistant); triticale (susceptible).
(d) Assay type: conical tube assay, visual assessment 21 days after sowing; infection score (0-5).
(e) Experiment design: 5 tubes / accession / plant sp.

The results of this experiment are presented in Table 1.

TABLE 1: Reaction of various *Triticum dicoccoides* accessions to *Gaeumannomyces graminis* var. *tritici*, isolate Galon '87.

PLANT SPECIES & ACCESSION NUMBER	No. OF PLANTS	INFECTION SCORE (0-5)	RESISTANCE RANKING
Triticum dicoccoides			
G 111-1-1	3	3.0	S
G 111-219	4	3.0	S
G 111-3M	5	3.3	S
G 114-2M	4	3.5	S
G 114-4M	5	3.3	S
G 114-5M	5	2.8	S
G 164	5	3.4	S
G 164-1	5	3.1	S
G 204-M	4	2.5	S
G 204-M-1	4	3.0	S
G 219-M	2	2.9	S
G 219-2M	3	2.2	S
G 219-3-2	4	3.3	S
G 232-MB	4	3.2	S
G 232-1	2	2.1	S
G 232-2	3	1.0	R
G 236-M	2	2.2	S
G 236-1	3	3.6	S
G 244-M	3	3.1	S
G 237-M	2	2.8	S
G 237-1B	3	3.1	S
G 237-2	5	1.6	R
G 237-8	5	2.5	S
G 315-M	1	2.8	S
G 315-2M	4	3.2	S
G 315-6M	1	2.7	S
G 315a-1M	3	2.4	S

TABLE 1: continued

PLANT SPECIES & ACCESSION NUMBER	No. OF PLANTS	INFECTION SCORE (0-5)	RESISTANCE RANKING
Triticum dicoccoides			
G 335-M	4	2.7	S
G 336-M	3	2.9	S
G 337-M	3	0.8	R
G 338-M	2	1.6	R ?
G 339-M	4	2.2	S
G 340-3M	2	2.1	S
G 341-M	2	3.1	S
G 342-M	4	1.4	R
G 343-M	4	2.5	S
G 344-M	2	2.1	S
G 345-M	3	2.8	S
G 119-MB	2	2.7	S
G 137-1M-1	4	2.9	S
G 333-M	2	2.7	S
WHEAT	5	2.9	
BARLEY	5	2.2	
RYE	5	2.4	
TRITICALE	5	2.0	

The results indicate that four accessions showed some resistance. However, both the small number of replicates and the large variability between individual plants, calls for further testing.

1b: Screening Portuguese cultivars of Triticum aestivum and aliens

Genetic resources of wheat and wild relatives of wheat of Portuguese flora were received from the National Plant Breeding Station at Elvas. This screening program is conducted by using the pot method with Setaria inoculum. Two levels of inoculum, 40 and 350 propagules /kg were selected for this test. Results are under way.

2: Reproducibility and consistency of reaction of T. dicoccoides accessions inoculated with 4 GGT isolates

(a) GGT isolates: Bet-Kama, Harel 4, Kfar Menachem, Galon '87.

(b) Inoculum types: PDA disks.

(c) Test plants: 8 accessions of T. dicoccoides that were assessed in previous experiments were selected for this assay. The first 3 accessions were previously ranked as susceptible, and 5 entries have shown resistance. The Standards:wheat cv. "Barkai" (susceptible); barley "DRO 32" (intermediate); rye "Wesser" (resistant); triticale (susceptible).

(d) Assay type: Conical Tube assay, visual assessment 21 days after sowing; infection score (0-5).

(e) Experiment design: 5 tubes / accession / plant sp.

The results of this experiment are presented in Table 2.

TABLE 2: Reaction of various Triticum dicoccoides accessions to four isolates of Gaeumannomyces graminis var. tritici.

PLANT SPECIES & SERIAL ACCESSION	ISOLATE	No. OF PLANTS	INFECTION SCORE (0-5)
T. dicoccoides	Bet Kama		
G 333-3	"	5	2.1
G 137-1M-1	"	5	2.7
G 119-2	"	5	3.0
G 342-2-2-3-6-1	"	5	2.5
G 338-1	"	5	2.6
G 337-4	"	5	2.8
G 237-2	"	5	2.8
G 232-4	"	5	2.7
WHEAT	"	5	2.6
RYE	"	5	2.4
BARLEY	"	5	2.3

Table 2: continued

PLANT SPECIES & SERIAL ACCESSION	ISOLATE	No. OF PLANTS	INFECTION SCORE (0-5)
T. dicoccoides	Harel 4		
G 333-3	"	5	2.8
G 137-14-1	"	5	2.7
G 119-2	"	5	2.9
G 342-2-2-3-6-1	"	5	2.4
G 338-1	"	5	2.6
G 337-4	"	5	2.5
G 237-2	"	5	2.6
G 232-4	"	5	2.6
T. dicoccoides	K. Menachem		
G 333-3	"	5	2.4
G 137-14-1	"	5	2.7
G 119-2	"	5	2.9
G 342-2-2-3-6-1	"	5	2.7
G 338-1	"	5	2.3
G 337-4	"	5	2.2
G 237-2	"	5	2.4
G 232-4	"	5	2.4
WHEAT	"	5	2.4
RYE	"	5	1.6
BARLEY	"	5	2.0
TRITICALE	"	n.t.**	n.t.**

Table 2: continued

PLANT SPECIES & SERIAL ACCESSION	ISOLATE	No. OF PLANTS	INFECTION SCORE (0-5)
T. dicoccoides	Galon '87		
G 333-3	"	5	2.2
G 137-14-1	"	5	2.7
G 119-2	"	5	2.7
G 342-2-2-3-6-1	"	5	2.7
G 338-1	"	5	2.4
G 337-4	"	5	2.0
G 237-2	"	5	2.9
G 232-4	"	5	2.5
WHEAT	"	5	1.9
RYE	"	5	1.4
BARLEY	"	5	1.7
TRITICALE	"	5	2.8

" n.t.= not tested

The results do not confirm previous indications of resistance. Moreover, in this series of assessments, the reaction spectrum of the standards was unusually narrow, thereby limitting the ability of a moderate degree of resistance to be manifested. Therefore, from this series of tests we cannot conclude about the reproducibility and consistency of reaction of unknwn accessions screened for resistance.

3: Assessing aggressiveness of six Israeli GGT isolates

(a) GGT Isolates: (1) Bet-Nir; (2) Harel; (3) Netiv HaLamed-He; (4) Lahav; (5) Galon; (6) Kfar Menachem

(b) Inoculum Type: OKM

Oat kernel medium (OKM): 0.25L (vol) oat kernels + 200ml tap water, autoclaved twice for 30min each time in a 1L Ehrlenmeyer flask, are inoculated with PDA sqares from one 9cm Petri-dish filled by a GGT colony and incubated at 29 C in darkness until all kernels are well

colonized (3-4 weeks). Colonized kernels are air-dried in ethanol-disinfected trays, hermetically covered by two layers of etnanol-disinfected tissue-paper, under a continuous flow of compressed air filtered through sterile 0.45μ filters. Dried kernels are ground for 3 seconds (dry) in a Waring blender and fractionated through sieves into three fractions: < 0.5mm; 0.5-2.0mm; > 2.0mm. Whole dried kernels and separate fractions are stored at room temperature.

c) Tested Species: Wheat cv. "Barkai" (susceptible standard).

d) Assay Type: conical tube assay, inoculated with 2 oat kernels/tube. Disease index 0-5.

e) Experiment Design: 5 tubes (1 plant/tube) =5 replicates/isolate, each replicate is an average of 4-6 roots, results given as av. of 5 replicates in Table 3.

TABLE 3. A comparison of infection scores of wheat, cv. "Barkai" artificially inoculated with six Israeli isolates of *Gaeumannomyces graminis* var. *tritici*

ISOLATE	INOCULUM TYPE	DISEASE INDEX (INFECTION SCORE)
1) Bet-Nir	(OKM)	2.5
2) Harel	(OKM)	2.75
3) Netiv HaLamed-He	(OKM)	3.73
4) Lahav	(OKM)	2.33
5) Galon	(OKM)	1.6
6) Kefar Menahem	(OKM)	4.25

The relative aggressiveness of these Israeli GGT isolates, as shown in Table 3, is very similar to that reported in the previous report, where the inoculum type was PDA disks. This indicates that differences in aggressiveness are measurable by the conical tube assay. The most aggressive isolate was Kefar Menahem, and the least aggressive was Galon.

4: Assessing aggressiveness of 18 Portuguese GGT isolates

(a) GGT isolates: 18 isolates collected during May-June 1988 in the most important cereal growing region in Portugal, Alentejo (south of Lisbon).
(b) Inoculum Type: PDA disks, 12 mm diam.
(c) Tested Species: wheat cv. "Anza"
(d) Assay Type: conical tube assay (30 mm diam., 3 seeds/tube; disease index: compound - root discoloration + runner hyphae + aerial symptoms).

Only 8 isolates were regarded as pathogenic. The other isolates colonized the roots thoroughly with runner hyphae, but did not cause any detectable root discoloration or aerial symptoms and were therefore regarded as non-pathogenic. The plants infected by the latter isolates remained green for the period of incubation. We are not yet in a position to establish whether those non-pathogenic isolates are GGT or *Phialophora* sp.

II. THE EFFECTS OF PLANT INTERACTIONS WITH INOCULUM TYPES AND DOSAGES AND THE EFFECTS OF INFECTION DEGREE ON PLANT GROWTH AS RESISTANCE ASSESSMENT METHODS

Methodology: Preparation of Various Inoculum Types

I. Wheat Straw Vermiculite (WSV): Air-dried, brittle wheat straw is cut with scissors into fine pieces. Equal volumes of straw (50ml = 7.7g) and vermiculite (50ml = 8.3g) are mixed and soaked overnight in water (70ml + 100mg NH_4NO_3). Soaked mixture is autoclaved twice for 30min. The medium is inoculated with squares of a PDA culture of GGT and incubated at 29 C until uniform colonization of the medium is complete.

II. Maize Meal Vermiculite (MMV): Aliquots of 35ml of maize meal and vermiculite (=1.5g maize meal + 6g vermiculite) were moistened with 15ml water and autoclaved 2 x 20 min in 150ml Ehrlenmeyer flasks. Inoculation and incubation as described above.

III. Maize Meal Quartz (MMQ): Aliquots of 34ml (=1.5g maize meal + 48.5g rough quartz sand) were moistened with 10ml water and autoclaved 2 x 20 min in 150ml Ehrlenmeyer flasks. Inoculation and incubation as described above.

IV. Millet Seed Medium (MSM): 20g seeds are washed well under a stream of tap water, on a screen, until no more dirt is visible in the water, seeds are then soaked overnight in tap water. Soaked seeds are rinsed again under a stream of water and boiled slowly for 30 min, starting with a total volume of 100ml and finishing with 50g of boiled seeds, all water being absorbed or evaporated. Autoclaving, inoculation and incubation as described above.

V. Oat Kernel Medium (OKM): 0.25L (vol) oat kernels + 200ml tap water, autoclaved twice for 30min each time in a 1L Ehrlenmeyer flask, are inoculated with PDA sqares from one 9cm Petri-dish filled by a GGT colony and incubated at 29 C in darkness until all kernels are well colonized (3-4 weeks). Colonized kernels are air-dried in ethanol-disinfected trays, hermetically covered by two layers of etnanol-disinfected tissue-paper, under a continuous flow of compressed air filtered through sterile 0.45µ filters. Dried kernels are ground for 3 seconds (dry) in a Waring blender and fractionated through sieves into three fractions: < 0.5mm; 0.5-2.0mm; > 2.0mm. Whole dried kernels and separate fractions are stored at room temperature.

VI. Setaria Seed Medium (SSM): Seeds of Setaria italica are soaked in water overnight. Excess water was quickly drained and the seeds were autoclaved in Ehrlenmeyer flasks for 30 min daily, for three consecutive days. Each sterilized flask was inoculated with PDA disks cut from the growing edge of GGT colonies. Flasks were incubated for one month at 25 C. Seeds were air-dried after incubation as described above. No grinding was necessary.

5: Assessing the potential of five inoculum types in Conical Tubes

(a) GGT Isolate: (1) Bet-Nir; (2) Harel; (3) Netiv HaLamed-He; (4) Lahav; (5) Galon; (6) Kfar Menachem

(b) Inoculum Types: OKM, MSM, WSV, MMV and MMQ.

(c) Tested Species: Wheat cv. "Barkai" (susceptible standard).

(d) Assay Type: conical tube assay, inoculated with 2 oat kernels/tube or with an approximately equal volume of the other inoculum types,

Disease index 0-5.

(e) Experiment Design: 5 tubes (1 plant/tube) =5 replicates/inoculum type or isolate, each replicate is an average of 4-6 roots, results given as av. of 5 replicates in Table 4.

TABLE 4. A comparison of infection scores of wheat, cv. "Barkai" artificially inoculated with five inoculum types of *Gaeumannomyces graminis* var. *tritici*, isolate 'Bet-Nir'

ISOLATE	INOCULUM TYPE	DISEASE INDEX (INFECTION SCORE)
Bet-Nir	(OKM)	2.50
- " -	(MSM)	2.96
- " -	(WSV)	0.81
- " -	(MMV)	1.83
- " -	(MMQ)	1.64

The results of this experiment seem to indicate that the inoculum types called MSM and OKM are the most potent of the five tested. As OKM is easier to prepare, most of the following experiments done with particulate inocula were carried out with OKM.

6: Assessing the potential of five inoculum types on plant development

(a) Isolates: Bet-Nir
- " -
- " -
- " -
Harel

(b) Inoculum Type: (I) (WSV)
(II) (MMV)
(III) (MMQ)
(IV) (MSM)
(V) (OKM)
CONTROL (uninoculated)

(c) Tested Species: wheat cv. "Barkai" (susceptible standard); barley "DRO 32" (intermediate); rye "Explorer" C.I.15 (resistant standard).

(d) Assay Type: Square plastic pots, 800 ml vol. were used, with 9 pregerminated (48h) seeds planted/pot. Pot mixture consisted of a

2:1 (v/v) mixture of red sandy loam and vermiculite. Pots were tagged and filled with 350 ml pot mixture; 40 ml inoculum mixture were added as a 1cm layer and the pots were filled up to 650ml with pot mixture. Inoculum mixture: 680ml pot mixture + 90ml inoculum grown in Ehrlenmeyer flasks - mixed well.

Immediately before sowing pots were irrigated to ~75% field capacity = 150ml. Additional fertigation was provided once every 5-7 days, 45ml/pot, containing 0.5g/L "20:20:20" (commercial fertilizer containing P, K, N, and microelements).

Plants from 1 pot/plant sp./ treatment (except Controls) were washed out of the pots 32 days after planting and their roots were visually assessed for infection (infection score 0-5). The stems and leaves, together with those of ca. 3 plants cut at soil level from each of the other pots (total 13-23 plants/ plant sp./ treatment) were given individual serial numbers, weighed (fresh weight), oven-dried (90°C, 48 hr) and the dry weight/plant was recorded. All the results were statistically analysed (Duncan's multiple range test).

The remaining plants/pot will be compared as grain yields.

e) <u>Experiment Design</u>: 3 plant species; 6 treatments (5 inoculum types + uninoculated control); 6 pots/plant sp./treatment = 54 plants/sp /treatment. Total 108 pots.

The rsults are presented in Table 5.

TABLE 5. Effects of five Gaeumannomyces grminis var. tritici inoculum types on the dry weight, infection score (0-5), and percentage of infected roots of wheat, barley and rye.

TREATMENT/ PLANT SP.	n PLANTS	MEAN DRY WEIGHT (mg)	RELATIVE DRY WT. (% of contr)	DUNCAN GROUPING	INFECTION SCORE (0 - 5)	INFECTED ROOTS (% of total)
Wheat Control	16	88.3	100.0	A **(A)***	0	0
W (I)	8	71.4	80.9	B	4.56	16/16= 100
W (V)	14	61.9	70.2	BC	4.27	12/14= 86
W (IV)	13	56.2	63.6	BC	4.32	19/20= 95
W (II)	13	54.1	61.3	BC	3.35	18/19= 95
W (III)	10	52.0	58.9	C	4.20	26/26= 100
W (all trs.)	**58**	**58.5**	**66.3**	**(B)***	**4.18**	**91/95= 96**
Barley (C)	18	87.8	100.0	A **(A)***	0	0
B (IV)	23	82.7	94.3	AB	2.53	33/36= 92
B (II)	23	70.4	80.2	BC	3.97	32/38= 84
B (III)	22	69.9	79.6	BC	2.72	34/43= 79
B (I)	15	67.1	76.4	BC	3.69	30/40= 75
B (V)	23	63.6	72.5	C	2.17	32/41= 78
B (all trs.)	**106**	**71.6**	**81.6**	**(B)***	**2.98**	**161/198= 81**
Rye Control	15	82.7	100.0	A **(A)***	0	0
R (II)	16	80.6	97.5	AB	1.94	14/29= 48
R (III)	20	75.9	91.9	AB	0.84	17/35= 49
R (I)	15	72.3	84.4	AB	1.62	12/38= 32
R (IV)	17	69.3	83.8	AB	1.78	20/32= 63
R (V)	21	62.2	75.3	B	1.64	23/36= 64
R (all trs.)	**89**	**71.7**	**86.7**	**(A)***	**1.55**	**86/170= 51**

Treatments with different letters are significantly different ($P \leq 0.05$).
* All treatments pooled, compared with the control (T-test)

The results of this experiment indicate the following: (a) The decreases in dry weight caused by the five different inoculum types

were not significantly different, with very few exceptions. However, when all five inoculum treatments are pooled together and compared to the controls, the dry weight of the inoculated plants was significantly lower for wheat and barley and lower but not significantly so for rye. The overall tendency is the one known from our previous report: wheat, the most susceptible, yielded dry weight **66.3%** of the control (non inoculated), followed by barley (moderately sosceptible) (**81.6%**), and closing with rye (resistant) (**86.7%**) and not significantly different from the drt weight of the control (**100.0**); (b) The same tendency is evident when the visual infection scores are considered: wheat =**4.18**, barley =**2.98**, rye =**1.55**; (c) The percentage of infected roots shows again the same tendency: wheat **96%**, barley **81%**, rye **51%**. Again, the differences in infection scores and in the percentage of infected roots, between the different inoculum treatments, were small and probably not significantly different.

7: Dose-response experiment with SSM

(a) GGT Isolates: several Portuguese isolates.

(b) Inoculum Type: Setaria Seed Medium (SSM) inoculated simultaneously with several Portuguese isolates to ascertain pathogenicity in the event of pathogenicity loss by individual isolates.

(c) Test Plants: wheat cv. "Anza".

(d) Assay Type: Plastic pots, 80 mm upper diam. x 155 mm height (Fig. 3) were filled with 500 g of soil just infested and were watered immediately to approx. 60% field capacity. Pregerminated seeds were sown several hours later, 6 seeds/pot, (to allow uniform water distribution) in 1 cm deep holes and were futher covered by a 1 cm deep layer of wet sand. Pots were incubated for 5 weeks in a controlled environment chamber set at 19±2 C, with a 15 h light photoperiod and 75% RH.

Infested soil was prepared by thoroughly mixing 0 (Control), 20, 40, 80, 160, 320 and 640 colonized seed propagules (Setaria) per kg of air-dried soil.

After 5 weeks seedlings were washed out of the pots and the roots were washed further under a gentle stream. Shoots were separated from the roots, their fresh weight was recorded, after

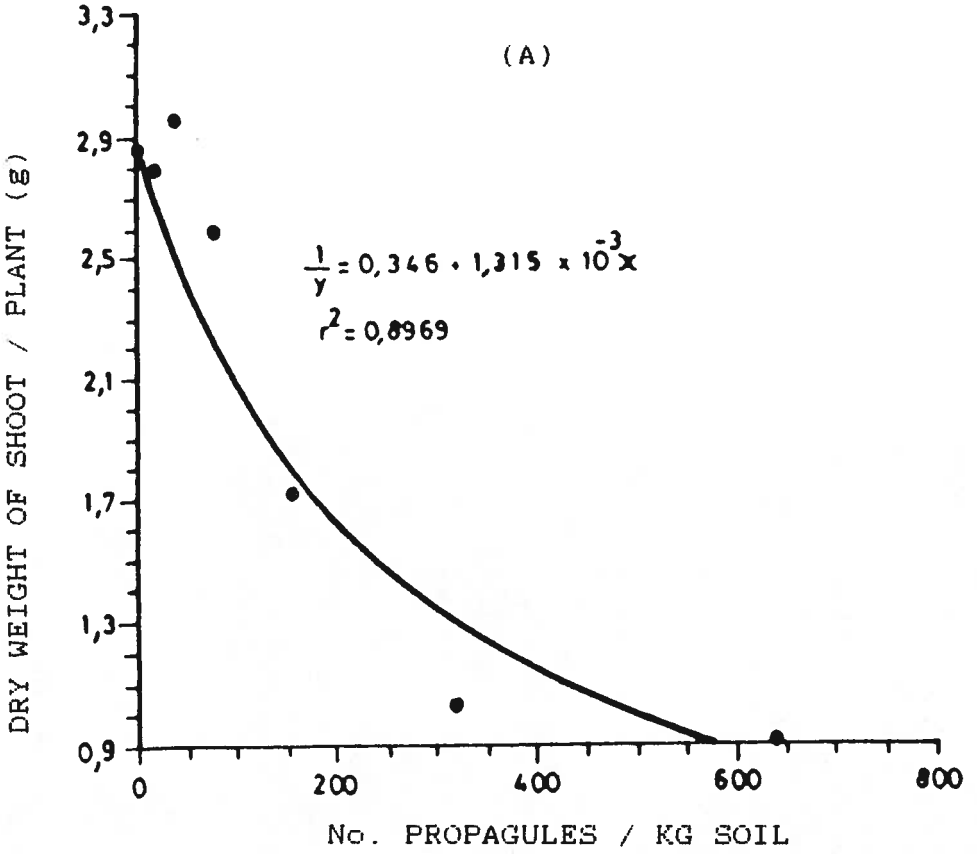
which they were air dried for 48 h at 100 C and their dry weight was recorded. The roots were preserved in 70% ethanol for subsequent recording of the number of discolored roots/plant.

(e) Experiment Design: 6 plants / pot; 10 pots / inoculum dose; 7 inoculum concentrations (control included). Total 70 pots.

Fig. 1 shows dose-response curves with very high negative correlations between inoculum dose and plant fresh and dry weight. Fig. 2 shows a similar dose-response curve with high correlation between inoculum concentration and the number of infected roots. Statistical analysis (Multiple range analysis, 95% LSD intervals) presented in Table 6 shows that the dry weight of plant shoots gave the highest inverse correlation with inoculum concentration.

TABLE 6: Multiple range analysis for shoot fresh weight, dry weight and average root discoloration as a response of wheat to increasing levels of GGT inoculum (95% LSD intervals).

PROPAGULES / KG SOIL	NO. OF POTS	FRESH WEIGHT (mg)	HOMOG. GROUP	DRY WEIGHT (mg)	HOMOG. GROUP	INFECTED ROOTS (av.)	HOMOG. GROUP
0	10	2860	A	464.7	A	0	A
20	10	2790	AB	450.6	A	25.4	B
40	10	2960	A	449.3	A	53.5	C
80	10	2590	B	440.7	A	79.0	D
160	10	1720	C	336.3	B	84.4	E
320	10	1022	D	256.7	C	86.8	EF
640	10	919	D	235.0	C	90.7	F



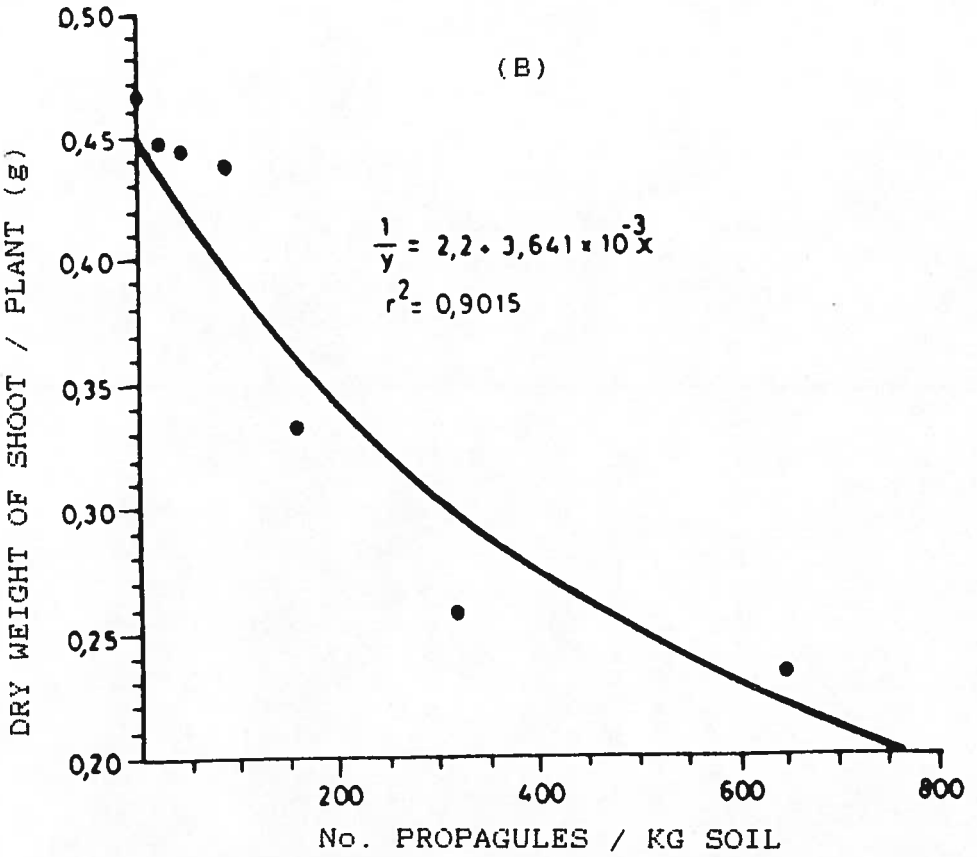


Fig. 1 Effect of increasing number of propagules on the shoot fresh weight (A) and dry weight (B).

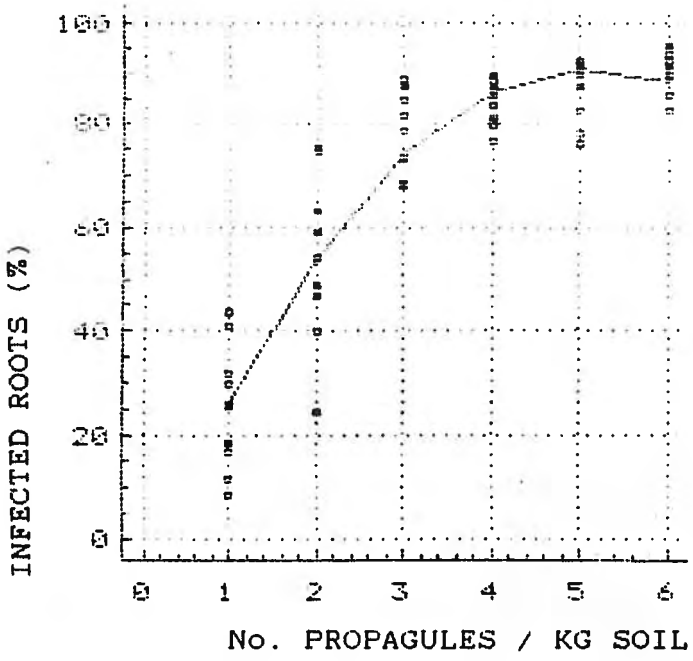


Fig. 2 effect of increasing number of propagules on the percentage of infected roots.
$y = -8.802 + 39.0045x - 3.8075$; $r = 0.8638$

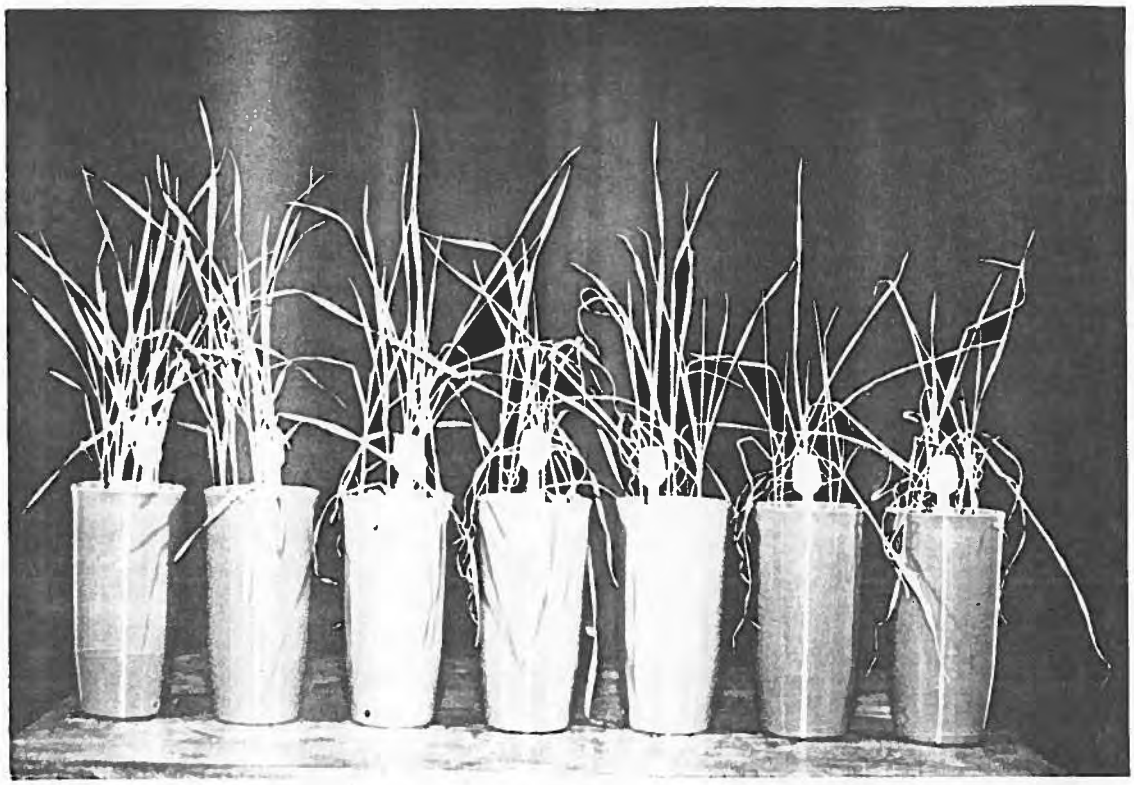


Fig. 3 Aerial symptoms of plants in the dose-response experiment (no. 7).

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8: Dose-response experiment with OKM

a) GGT Isolate: Harel b) Inoculum Type: Oat kernel medium (OKM) ground and fractionated through sieves: fraction 0.5-2.0mm was used as inoculum in the following concentrations:

2g/L: 17g OKM were thoroghly mixed by hand in 1L pot mix, which was further mixed 10 min in a concrete mixer with 7.5L pot mix.

1g/l: 7.2g OKM in 1L pot mix, mixed 10 min with 6.2L pot mix.

0.5g/L: 3.6g OKM in 1L pot mix, mixed 10 min with 6.2L pot mix.

0.25g/L: 0.9L(2g/L) mixed 10 min with 6.3L pot mix.

0.11g/L: 0.4L(2g.L) mixed 10 min with 6.8L pot mix.

UNINOCULATED CONTROL: pots filled with uninoculated pot mix

c) Test Plants: wheat cv. "Barkai" (susceptible standard); barley "DRO 32" (intermediate); rye "Explorer" C.I. 15 (tolerant std.)

d) Assay Type: in 800 ml square plastic pots filled with 500 ml inoculated pot mix and then up to 650 ml with uninoculated pot mix (2:1 v/v red sandy loam : vermiculite). Seeds pregerminated for 48h were planted, 9/pot, 1.5cm below surface. One month after sowing half of the plants in each pot were cut at ground level, attributed individual numbers and oven-dried at 90 C for 48h. The dry weight was recorded and the results were statistically analysed (Duncan's multiple range test, $P \leq 0.05$).

e) Experiment Design: 9 plants of each species/pot, 4 pots/plant sp./ treatment, Total 72 pots.

The results of this experiment showed no significant differences between the treatments (inoculum concentrations) and the controls, due to too great intra-treatment variability in plant size and plant dry weight. This could have been a result of great variability in seed weight. As a consequence the next experiments will be carried out with pre-weighed seeds which will be in the range of $\pm$ 10mg group.

III. A MODIFIED SLANT BOX METHOD FOR POINT INOCULATION EXPERIMENTS AS AN ASSESSMENT METHOD OF PLANT RESISTANCE TO GGT

9: Point inoculation (a)

a) GGT Isolate: Bet-Nir

b) Inoculum Types: (1) OKM 0.5-2.0 mm, (2) MMQ individual grains.

c) Test Plants: wheat cv. "Barkai" (susceptible standard); barley "dro 32" (intermediate); rye "Explorer" C.I.15 (tolerant std.).

d) Experimental Procedure: transparent plastic sleeves, 17.0 x 4.5cm, filled with vermiculite, watered to field capacity (ca 40 ml), with drainage hole cut at the bottom, 3-4 sleeves placed in black plastic pots held inclined (45°) in trays, to cause root growth along the lower walls of sleeves. One seed/bag. Inoculation was performed on each of 2-6 roots of each plant, at a distance of ca. 4 cm below the seed, by a small incision in the plastic and insertion of 1 grain of inoculum in contact with the root. 15 plants of each species were inoculated with each inoculum type. **Started:** 1st Dec 1988; **Inoculation:** 8 Dec 1988.

e) Assay Procedure: Inoculated roots are kept under microscopic surveillance. With the appearance of infection symptoms, measurements of the root length covered with hyphae, discoloration and lesions were taken every two or more days. **Assay dates:** 12/16/1988; 12/18; 12/20; 12/23; 12/28; **final:** 1/16/1989.
Results of the last of 6 readings are summarized in Table 7.

10: Point inoculation (b)

This experiment was carried out using the methods outlined in Exp. 9 (above), with the following modifications:

Started: 23 Jan 1989

Inoculation: (millet seeds (MSM), isolate: Kfar Menachem 87) 30 Jan 1989 Preliminary check, 8/2, showed very little infection, therefore a second inoculation was carried out.

Second Inoculation (OKM 0.5-2.0mm, isolate: Bet-Nir) 10 Feb 1989

Replicates: 15 slanted plastic sleeves (1 plant/sleeve) for each plant species; 2-5 roots inoculated/plant; sleeves filled with vermiculite.

Assay dates: **1**[19/2] **2** [24/2] **3** [] **4** [] **5** [] FINAL CHECK **6** []

Disease Assay: (Millimetres of discolored root)
The results of the last two readings are presented in Table 7.

TABLE 7. Comparative effects of *Gaeumannomyces graminis* var. *tritici* artificially inoculated to defined points on the seminal roots of susceptible wheat, moderately susceptible barley, and tolerant rye. In exp. 9 each inoculation point was inoculated once with with OKM. In exp. 10 each inoculation point was inoculated once with MSM and again after 11 days with OKM

PLANT SPP.	INOCULATED PLANTS	INOC. ROOTS	INFECTED ROOTS	AV. LESION LENGTH* (mm)	INFECTION** COEFFICIENT	DISEASE INDEX***	REL. DIS. ****INDEX (%)
Exp. 9							
Wheat	14	32	26	45.65	0.8125	37.1	100.0
Barley	30	76	41	27.07	0.54	14.6	39.4
Rye	15	30	6	31.5	0.2	6.3	17.0
Exp. 10							
Wheat	15	46	42	12.8	0.913	11.7	100.0
Barley	15	48	32	6.9	0.667	4.6	39.3
Rye	15	42	13	6.8	0.310	2.1	17.9

* Average Lesion Length includes only infected roots
** Infection Coefficient= infected roots/inoculated roots
*** Disease Index= av. lesion (mm) x Infection Coefficient
**** Relative Disease Index (%) when the wheat index is considered 100%

Despite the differences in the experimental design between experiments 9 and 10, the results, as relative disease index, are very similar, showing again the resistance of rye and the moderate susceptibility of in comparison to the susceptibility of wheat. The infection coefficients of the three plant species are also very similar in the two experiments, and correlate well with the percentage of infected roots obtained in experiment 6 (transformed into infection coefficients). This comparison is presented below:

	INFECTION COEFFICIENTS		
	Exp. 6	Exp. 9	Exp. 10
WHEAT	0.96	0.81	0.91
BARLEY	0.81	0.54	0.67
RYE	0.51	0.20	0.31

The point inoculation method turns out to be a very precise one, where every infection is followed up from its inception, throughout all of its visible stages. For resistance screening purposes, this method requires more labor per plant assayed than other methods tested.