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Short Communication

Emmer wheat, a potential new host of *Tilletia indica*

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Abstract

Two representative Italian emmer wheat (*Triticum dicoccum*) landraces, two selected lines and three improved emmer wheat cultivars, derived from crosses with durum wheat (Molisano landrace × 'Simeto'), were tested for their susceptibility to *Tilletia indica*, the cause of Karnal bunt of wheat. Plants of emmer wheat were inoculated by injecting allantoid sporidial suspensions into the boot cavity of plants, just prior to ear emergence. A highly susceptible Indian spring wheat cultivar (*Triticum aestivum*) was used as a comparative control. At maturity of the plants, the seeds were harvested and assessed for incidence and severity of disease. All emmer wheat genotypes tested were infected but showed differing levels of susceptibility. The percentage of infected seeds for individual genotypes ranged from 5.4 to 75.0% compared with 99.1% for WL-711. The severity of infection was less in the old landraces, but it was higher in all the improved emmer wheat cultivars. In conclusion, Italian cultivars of emmer wheat were found to be highly susceptible to *T. indica*, and are potentially able to support the establishment of the pathogen.

Abbreviations: C.I. – coefficient of infection; PRA – pest risk analysis

The fungus *Tilletia indica* causes the disease Karnal bunt, or partial bunt, of bread wheat (*Triticum aestivum*), durum wheat (*Triticum durum*) and triticale (×*Triticosecale*). Seeds are infected through the germinal end of the grain and the fungus develops within the pericarp where it produces a powdery, brownish-black mass of teliospores. The presence of only 3% of infected grains reduces the quality, potentially leading to downgrading of milling wheat to feed wheat, due to the foetid, decaying fish-like smell (trimethylamine) produced by the spore masses.

Because of its potential for entry, establishment and economic damage in the EU, as assessed by the process of pest risk analysis (PRA), (Sansford, 1996, 1998), *T. indica* was added as a quarantine pest to the EC Plant Health Directive 77/93/EEC (now 2000/29/EC as amended) in 1997 (Anon., 2000). The EC Directive applies quarantine requirements to seed and grain of *Triticum, Secale* and ×*Triticosecale* from countries where *T. indica* is known to occur (currently listed in the EC Directive as: Afghanistan, India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and the USA). As rye (*Secale cereale*) is no longer considered to be a natural host, it is to be considered for deletion from the EC Plant Health Directive. A 4-year EU Fifth Framework Project with nine Partner Organisations in seven countries including Australia and the USA (http://karnalpublic.pestrisk.net) has developed a new PRA for *T. indica*. This concludes

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Table 1. Rating scale used to assess wheat cultivars for severity of Karnal bunt

Infection category	Symptoms	Assigned value for calculation of C.I.
0	Healthy	0
1	Well developed point infection, *c*. 25% seed bunted	0.25
2	Infection spreading along the groove, *c*. 50% seed bunted	0.5
3	Three-quarters of seed converted to sorus, *c*. 75% seed bunted	0.75
4	Seed completely converted to sorus, *c*. 100% seed bunted	1.0

that *T. indica* still has the potential to enter, establish and have a serious impact in the EU (C. Sansford, CSL, UK pers. comm.).

In order to evaluate the host range of *T. indica* to wheat other than *T. aestivum* and *T. durum*, different genotypes of *Triticum dicoccum* (emmer wheat), so far not known as a natural or potential host of *T. indica*, were tested for resistance/susceptibility in quarantine controlled environment rooms.

Cultivation of emmer (hulled wheat) is increasing in all of central and southern Italy as an environmentally friendly cereal grain suitable for organic farming. The landraces of emmer wheat from central Italy have predominantly a vitreous fracture kernel (like durum wheat) suitable for producing pasta, while those from southern Italy and Toscana Region generally have a floury fracture kernel (soft type) suitable for whole, semi-pearl and pearl grains.

Seven emmer wheat genotypes were used in this experiment: two representative Italian old landraces, 'Garfagnana' and 'Leonessa'; two selected lines from the landrace Molisano, 'Farvento' and 'Molise'; and three improved cultivars derived from crosses with durum wheat (Molisano landrace × 'Simeto'), 'Mosè', 'Davide' and 'Padre Pio'. A highly susceptible Indian spring wheat cultivar WL-711 (*T. aestivum*) was used as a comparative control. The 'boot' inoculation method used in this experiment is the universally accepted standard method in wheat breeding programmes aimed at developing cultivars with resistance to *T. indica*. This is considered to give the most reliable indication of 'physiological' susceptibility to infection. The inoculum was prepared according to Bonde et al. (1996), using a mixture of sporidia derived from three populations of teliospores collected from Mexico, India and the USA. Ten plants for each wheat genotype were inoculated at the boot stage (GS 45; Tottman and Broad, 1987) by injecting 1 ml of spore suspension (50,000 allantoid sporidia ml^{-1} dH_2O) into the boot cavity. In the same way, ten plants were treated by injecting 1 ml of sterile distilled water, as a negative control. After inoculation, transparent polyethylene bags were placed over the plants to maintain a high level of humidity, and the plants were transferred into a growth chamber with a 12 h light cycle (fluorescent light with tungsten lamp supplement) at 20/18 °C (day/night) and 60% RH. The bags were removed after 3 days.

To favour the maturation of the wheat heads, the temperature in the growth chamber was raised to 23/20 °C (day/night) 4 days after inoculation and to 25/22 °C (day/night) 30 days after inoculation. The ears were collected at maturity (GS 92) and the seeds were assessed for the incidence of Karnal bunt, using an 'infection category' rating scale and 'susceptibility categories' based on those used by Aujla et al. (1989) and Bonde et al. (1996) (Tables 1 and 2 respectively). Susceptibility categories were based on 'coefficient of infections' (C.I.) (Table 2), a disease index calculated using the incidence and severity of disease for each wheat genotype (Table 3). All the processes described above were done in licensed quarantine containment facilities.

All of the genotypes of emmer wheat tested showed Karnal bunt symptoms. The percentage of infected seeds for individual emmer wheat cultivars ranged from 5.4 to 75.0% compared with 99.1% for the susceptible Indian spring wheat control 'WL-711' (Tables 3 and 4). For the

Table 2. Susceptibility categories for *T. indica* related to calculated coefficients of infection (C.I.)

C.I.	Susceptibility category
0	Highly resistant (HR)
0.1–5.0	Resistant (R)
5.1–10.0	Susceptible (S)
10.1–20.0	Moderately susceptible (MS)
20.1 and above	Highly susceptible (HS)

Table 3. Susceptibility of emmer wheat to *Tilletia indica* tested by 'boot' inoculation: incidence (% seeds infected) and coefficient of infection (C.I.) for each Italian emmer wheat genotype compared to a highly susceptible spring wheat Indian cultivar (WL-711)

Cultivar	Total % infected seeds	Total No. of infected seeds	Distribution of infected seeds by infection category (1–4)				C.I.	Susceptibility category
			1	2	3	4		
Garfagnana[a]	18.4	14	10	3	1	0	6.25	S
Leonessa[a]	18.9	18	16	2	0	0	5.26	S
Farvento[b]	5.4	2	2	0	0	0	1.35	R
Molise[b]	21.5	14	14	0	0	0	5.38	S
Mosè[c]	45.5	10	0	0	1	9	44.32	HS
Davide[c]	32.0	8	1	2	1	4	24.00	HS
Padre Pio[c]	75.0	9	0	0	3	6	68.75	HS
WL-711 control	99.1	116	2	11	36	67	85.53	HS

C.I. = [(0.25 × seeds in Cat.1) + (0.5 × seeds in Cat.2) + (0.75 × seeds in Cat.3) + (1.0 × seeds in Cat.4)] × 100 / total No. of grains (Cat. 0–4).
[a]Landrace genotypes.
[b]selected lines from the landrace Molisano.
[c]improved emmer wheat cultivars (Molisano landrace × 'Simeto').
HS: highly susceptible, S: susceptible, R: resistant.

improved emmer wheat cultivars Mosè, Davide and Padre Pio it ranged from 32.0 to 75.0%. These cultivars were classed as 'highly susceptible' (HS) based on their C.I., as was the Indian spring wheat control WL-711 (C.I. 85.53). The landraces Leonessa and Garfagnana and the selected line Molise were classed as 'susceptible' (S). Only 'Farvento', the other selected line from the landrace Molisano, was classed as 'resistant' (R) (Table 3).

Other wild wheat and grass species have been found to be susceptible to *T. indica* under experimental conditions (Royer and Rytter, 1988; Nagarajan et al., 1997; Peterson and Bonde, 1998). *T. dicoccum* has been included in a number of studies since 1940 as a potential source of resistance for Karnal bunt and some accessions are reported to be resistant (Singh et al., 1993; Wilcoxson and Saari, 1996). This is the first report of *T. dicoccum* as a potential host of *T. indica*.

The cultivation of emmer wheat is rapidly increasing in several Italian areas as one of the alternative cereals for human consumption and industrial transformation. The high susceptibility to *T. indica* of the emmer wheat cultivars tested in this research is being taken into account in a new

Table 4. Results of the assessment of seven emmer wheat genotypes artificially inoculated with *T .indica* (boot injection at Growth Stage 45) compared to a highly susceptible Indian spring wheat cultivar (WL-711)

Cultivar	No. heads infected	Total No. of seeds	Total No. of infected seeds	Total % infected seeds	Mean % infected seeds/head	Mean % infected seeds/infected head
Garfagnana[a]	3/9	76	14	18.4	16.8	50.5
Leonessa[a]	7/10	95	18	18.9	29.2	41.7
Farvento[b]	2/4	37	2	5.4	16.7	33.4
Molise[b]	6/10	65	14	21.5	21.0	35.0
Mosè[c]	2/3	22	10	45.5	66.7	100.0
Davide[c]	1/3	25	8	32.0	33.3	100.0
Padre Pio[c]	3/5	12	9	75.0	60.0	100.0
WL-711 control	12/12	117	116	99.1	99.5	99.5

[a]Landrace genotypes.
[b]selected lines from the landrace Molisano.
[c]improved emmer wheat cultivars (Molisano landrace × 'Simeto')

PRA for Europe (C. Sansford, CSL, UK pers. comm.) as they are also potentially able to support establishment of this EC-regulated quarantine pest. In addition, the results obtained in this work, even if the experiments have been conducted on a limited number of genotypes, seem to indicate a higher susceptibility for emmer crosses with durum wheat to *T. indica* than for genotypes belonging to the old landraces. Consequently, it could be hypothesised that the genetic basis of *T. indica* susceptibility can be transmitted to emmer wheat *via* crosses with durum wheat. The possibility that in the future *T. indica* could establish itself in Europe should be taken into account in breeding programmes for new emmer wheat cultivars.

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