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In vitro screening for resistance against *Septoria nodorum* blotch in wheat

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Abstract This study was carried out to develop an in vitro test for the identification of genotypes resistant to *Septoria nodorum* blotch. The basis for this project was a previous study in which a crude extract of *S. nodorum* was used as a selective agent (Keller et al. 1994). It was possible to distinguish resistant and susceptible cultivars in an in vitro test with zygotic embryos. In our project we wanted to test whether this in vitro test can also be used to detect resistant and susceptible genotypes in early segregating populations. Specific crosses between eight winter wheat lines showing contrasting resistance reaction for *S. nodorum* blotch on leaves and ears were made. The resistance level of both leaf and ear was evaluated after artificial inoculation in the field for the parental lines, the F_1 progenies, as well as for segregating F_3 and F_4 populations. In addition, this plant material was tested in vitro using methods similar to those described by Keller et al. (1994), i.e. culturing immature zygotic embryos and mature seeds on selective media. A good agreement between in vitro screening and field resistance on the ear was found for the parental lines, the F_1 and F_4 generation but not for the F_3 generations. This leads to the conclusion that the in vitro screening might be integrated into wheat breeding programs. Populations showing a high susceptibility to the pathogen metabolites in vitro could be discarded. Another promising implementation for wheat breeding would be the screening of advanced breeding material or candidate partners in a crossing program for resistance on the ear. However, the in vitro screening is not precise enough to select single plants in early segregating populations.

Key words In vitro selection · *Septoria nodorum* · *Triticum aestivum* L. · Crude extract · Toxin resistance

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Introduction

Septoria nodorum blotch (SNB) is a fungal disease of wheat (*Triticum aestivum* L.) causing leaf and glume blotch. It is a serious pathogen in many wheat-growing areas throughout the world and may reduce yields up to 50% (Eyal et al. 1987). The causal agent is *Leptosphaeria nodorum* E. Müller [= *Phaeosphaeria nodorum* (E. Müller) Hedjaroude], anamorph = *Septoria nodorum* (Berk.) Berk. in Berk. & Broome [= *Stagonospora nodorum* (Berk.) Castellani & Germano].

Alternative approaches to conventional breeding are based on marker-assisted breeding or plant tissue- and cell-culture (Pauls 1995). Using toxic metabolites produced by pathogens as selective agents in vitro, disease-resistant plants have been obtained in various host-pathogen systems. (Daub 1986, Van den Bulk 1991, Ahmed and Sagi 1993). In previous experiments, synthetic mellein, a metabolite produced by *S. nodorum*, showed no selective action to zygotic wheat embryos cultured in vitro (Keller et al. 1994). On the other hand, a crude extract of *S. nodorum* containing a mixture of toxins from the pathogen showed a clear selective action in vitro to wheat embryos originating from wheat varieties with different levels of partial resistance on the ear (Keller et al. 1994).

The aim of the present study was to determine whether this method can be applied to differentiate resistance levels of breeding material in early generations. Therefore, a large number of zygotic embryos in the F_1 and the segregating F_3 generations, as well as F_5 seeds from the segregating F_4 generation originating from 16 crosses, were screened in vitro. Crude extracts from *S. nodorum* containing metabolites of the pathogen were used as selective

agents. The different sensitivity to these toxins observed in vitro was compared to the resistance reaction of the corresponding genotypes in the field after artificial infection (Wicki et al. 1999). A good agreement between in vitro screening and field resistance on the ear was found for the parental lines, the F_1 and F_5 generations but not for the F_3 generation. Possible explanations and implementations of the in vitro methods for wheat breeding are discussed.

Materials and methods

Plant material

The same eight winter wheat cultivars and lines (Arina, Iena, Forno, SN-, SN+, Boval, Zenith, Greif) that were used in the inheritance study of Wicki et al. (1999) were selected as parents. SN+ and Greif showed a high level of resistance on the ear and the leaf, while Arina and Iena were resistant against SNB on the ear only and Boval against SNB on the leaf only. To test the in vitro selection in segregating populations, 16 crosses were made (Table 1) among the eight parental lines. The crosses were chosen in such a way that all combinations of a high and low level of resistance for SNB on the ear and the leaf occurred (Wicki et al. 1999). The F_1- and F_3-isolated immature zygotic embryos, as well as F_5 kernels of each population, were used for in vitro screening.

Preparation of *S. nodorum* extract

Autoclaved wheat kernels from the cultivar Arina were inoculated with a mixture of a broad spectrum of 140 isolates collected in Switzerland each year. Together with the same amount of autoclaved water in 250-ml Erlenmayer flasks, the mixture was multiplied first for 1 week at room temperature and then for 4 months at 4°C. The content was air dried and then milled, according to the description of Fried (1989). The resulting powder was well mixed. Crude extracts of *S. nodorum* were produced using a soxhlet apparatus (300 ml) and ethyl acetate as an extracting agent (Sachse 1992). After a 15-h extraction, ethyl acetate was evaporated under vacuum at 50°C in a rotovapor and remaining traces of ethyl acetate were removed by a stream of nitrogen. Five milliliters of water and 0.05% Tween 20 were added to the solution and put on a shaker overnight. Extracts were obtained by sterile-filtering the solution through a syringe. Extract solutions from ten extraction cycles were put together as one batch, giving about 50 ml of crude extract. One batch was prepared for the in vitro selection experiment of 1994 (batch 1). A second batch was prepared for the in vitro selection experiments in 1996 (batch 2). The second batch was produced according to the first batch with one exception: the soxhlet apparatus had a volume of 5000 ml, and the batch consisted therefore only of two extracts (150 ml).

In vitro test – embryo culture

For the in vitro screening ears were harvested in the field or greenhouse at growth stages 83 to 85. Caryopses of the collected ears were surface sterilised for 3 min in 95% ethanol. Immature zygotic embryos, about 2 mm long, were isolated under sterile conditions on a flow bench and transferred to in vitro culture. Embryo culture was induced on Murashige and Skooge (MS) medium (Murashige and Skooge 1962) with 30 g/l of sucrose, 2 mg/l of 2,4-D, 8 g/l of Difco Agar and 2 g/l of Phytagel. Embryos at this stage in culture show direct shoot and root formation. Selective media were prepared by adding crude extract from *S. nodorum* (batch 1) to the MS medium (for concentrations see below) at about 50°C and will be called MSN medium. Seven milliliters of medium were poured into 6-cm Petri dishes at about 50°C. When the medium was solid, 12 embryos per Petri dish were grown at 26°C with a light intensity of 60 $\mu Em^{-2}s^{-1}$ for 16 h per day. An assessment of germination was made after 7 to 10 days. The concentration for the best selectivity of the extract in the MS medium was evaluated by testing a concentration range of 0. 1:100 to 1:1400 (v:v) of extract (batch 1) in the medium. The variety Forno, with a low level of resistance to SNB on the ear, and the variety Arina, with high level of resistance of the ear, were used as standards. For each concentration and each genotype, 100 zygotic embryos were cultured. In each experiment, 200 embryos (100 on MS medium, 100 MSN medium) of the eight parental lines were cultured in the same way with two replications as a reference. Relative germination rates on selective medium compared to the control were calculated and transformed with log (x + 1), giving the in vitro index (VI).

Table 1 Sixteen specific crosses between eight parental cultivars and lines and the numbr of randomly selected individual F_2 plants per population

Population no.	Female parent	Male parent	No. of F_2 progenies
1	Zenith	Greif	23
2	Iena	Zenith	18
3	Iena	SN–	20
4	Iena	Forno	22
5	SN+	Zenith	21
6	SN+	Iena	22
7	SN+	Boval	23
8	Arina	Iena	21
9	Arina	SN–	18
10	Arina	Forno	24
11	SN–	Zenith	24
12	SN–	Greif	23
13	SN–	SN+	28
14	Forno	SN–	22
15	Boval	Zenith	20
16	Boval	Iena	23

In vitro test – culture of mature seeds

Whole kernels containing mature embryos were cultured in vitro in a germination dish (20 cm × 13 cm) containing three filter papers of the same size and 15 ml of H_2O. This treatment was used as a control medium. By adding crude extract (batch 2) to the water at concentrations 0 and 1:100 to 1:1400, the optimal concentration for selectivity was determined screening again the variety Arina, with a high level of resistance on the ear, and Forno, with high susceptibility on the ear. No surface sterilisation was necessary prior to culture. Germination was induced at 20°C and normal day light. One hundred seeds of each of the eight parents were placed on water-containing dishes, and 100 seeds on dishes containing crude extract at the optimal concentration (this treatment will be called selective medium). Then, 200 seeds (100 on control medium, 100 on selective medium) were cultured. Seeds of each parental line were cultured in the same way with two replications as a reference and the in vitro indices were calculated according to the embryo culture.

In vitro screening experiment 1, F_1

200 F_1 embryos from each of the 16 crosses were harvested from plants that were grown and crossed in the green house. One hundred embryos were cultured on MS medium and 100 embryos on MSN medium and the in vitro index (VI) was calculated.

In vitro screening experiment 2, F_3

In autumn 1993, F_2 seeds of all 16 crosses were sown in 6-row-plots of 1.2 m × 3,5 m. In spring 1994, 18–28 individual plants out

of each cross were selected randomly (Table 1) and dug up at growth stage 15 (Zadoks et al. 1974). They were planted into soil at a 20×30 cm space for each plant and labelled (352 in total). All ears except one of each individual plant were collected at growth stage 83–85. The remaining ear was left to produce seeds for field testing. Thirty to fifty embryos of the 352 selected plants from the 16 populations were cultured on MS medium without extract as a control as described above, and 30–50 on MSN medium containing the crude extract at the optimal concentration. In vitro indices were calculated as described above. Altogether, in this in vitro selection experiment, about 30 000 F_3 embryos were cultured in the two treatments.

In vitro screening experiment, F_4–F_5

In the meantime, each individual F_2 plant used for experiment 1 was propagated to five F_4 lines (5-row plot). From plots showing homogeneous resistance reactions between the five rows derived from different F_3 plants but the same F_2 plant, one line was harvested. From plots showing different resistance reactions between the 5 rows, the most-susceptible and the most-resistant row was harvested (550 in total). Then, 200 F_5 seeds from the selected F_4 rows (100 on control medium, 100 on selective medium) were cultured in vitro. In vitro indices were calculated as described above.

Field trials

Progenies of 16 different crosses as well as the parental lines were screened in the field for their resistance against *S. nodorum* leaf and glume blotch in Zürich-Reckenholz, Switzerland. The F_1 and F_3 progenies were sown as 1-row plots in the fall of 1995 and 1994 respectively. The F_4 progenies were sown as 5-row plots with three replications. In each field trial the parental lines were included as a reference. The trials were inoculated four times with a mixture of a broad spectrum of isolates collected in Switzerland (same isolates that were used for the production of the crude extracts) of 1 million viable spores per ml (400 l/ha, 20 ml per row). The first inoculation was applied at the booting stage (growth stage 47–49; Zadoks et al. 1974). The second to fourth inoculation was applied to the heads. In order to avoid interactions with other diseases the trial was sprayed with 1 l/ha of Tiptor (Maag, Switzerland) 6 weeks before inoculation with *S. nodorum*. Oat slug worms were controlled by spraying with 1.5 l/ha of Zolone (Maag, Switzerland) at growth stages 50–55.

Scoring of resistance level in the field

The percentage of necrosis on each of the top three leaf layers (5–7 times within 4 weeks, between growth stages 50 and 80) and the percentage of necrosis on the ear (4–7 times within 4 weeks between growth stages 60 and 85) were recorded. In order to obtain a normal distribution of the percentage of diseased area on the ear and leaf, each recording was transformed with log (x+1). For each line, transformed scores of all dates were added up. This resulted in the severity index SNAE for the ear and LNLF for the leaf, which was used to determine quantitative differences in the resistance reaction.

Comparison of thein vitro methods with the resistance reaction in the field

In vitro indices (VI) of the embryo culture and the seed culture were correlated to the recorded resistance levels (SNEA and SNLF) in the field. In vitro indices of the parental lines were used to determine the effect of the different batch 1 and batch 2 *S. nodorum* extracts and to compare the results of embryo culture with those of seed culture.

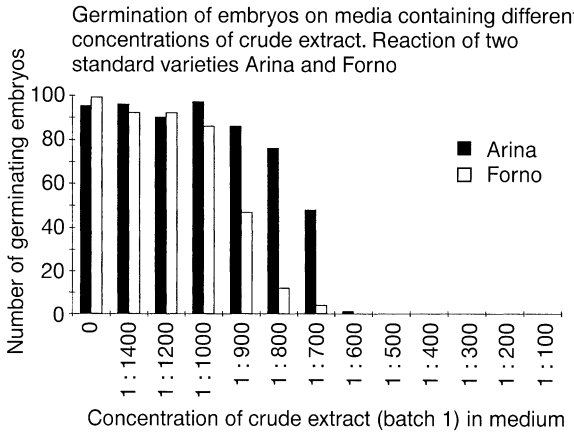

Fig. 1 Number of germinating embryos per 100 cultured embryos at different concentrations of crude extract of *S. nodorum* (batch 1) in the medium. Reactions of the variety Arina (high level of resistance on the ear) and the variety Forno (low level of resistance on the ear)

Results

Concentration range

By testing a concentration range of the crude extract of batch 1 in medium a clear selective action to wheat embryos was found for the two varieties Arina and Forno which showed different resistance reactions on the ear in the field. The number of germinating embryos per 100 cultured embryos is shown in Fig. 1 for each concentration of MSN medium and the MS medium. A selective action was observed between the concentrations 1:900 and 1:600. Higher concentrations were toxic for both genotypes, whereas lower concentrations did not reduce the germination rate of embryos. Within the selective range, the germination rate of the susceptible variety Forno was more reduced than that of the resistant variety Arina. For further experiments, a concentration of 1:750 was chosen for batch 1. Similar results were obtained by testing a concentration range but cultivating mature seeds instead of embryos and using batch 2. The concentration for the best selectivity in this case was1:350 (v:v).

In vitro screening of the parental lines

The in vitro screening of zygotic embryos using batch 1 differentiated the eight parents. Forno showed the most sensitive reaction to the extract in vitro and had an in vitro index (VI) of 0.03, whereas Arina showed the highest tolerance with a VI of 0.24. The numbers of germinating embryos per 100 cultivated embryos on MS medium and on MSN medium are given in Table 2. Almost the same results were obtained by cultivating mature seeds of the eight parental lines on control medium and on selective medium using batch 2. While on the control all varieties showed a germination rate of at least 96%, the range of

Table 2 Number of germinating embryos per 100 cultured embryos on control medium and on selective medium containing a crude extract (batch 1) of *S. nodorum*, together with the level of SNEA and SNLF resistance in the field in 1995 and 1996 (Wicki et al. 1999). Values of the eight parental lines

Variety	Control medium	Selective medium	VI	Resistance level in the field			
				SNEA 95	SNEA 96	SNLF 96	SNLF 96
Arina	99	73	0.24	5.50	3.06	27.54	23.51
Greif	95	59	0.21	5.47	3.14	19.95	18.34
Iena	98	60	0.21	4.88	3.54	24.16	21.94
SN+	91	43	0.17	5.93	3.64	22.19	20.14
Zenith	95	29	0.12	6.58	3.38	29.52	22.30
SN–	94	24	0.10	8.08	4.36	30.54	25.23
Boval	98	19	0.08	8.82	5.59	24.79	20.53
Forno	99	8	0.03	8.40	4.78	27.23	22.31

VI on the selective medium was between 0.07 (19%, Forno) and 0.18 (51%, Arina). There was the same rank order for VI compared to the embryo – system, and the correlation between the two systems was very high ($r = 0.93$, $P < 0.01$).

In vitro screening of F_1 zygotic embryos

Germination rates of F_1 embryos on MS control medium reached 100/100 over all crosses. VI on MSN medium of batch 1 ranged from 0.01 (3/100, Boval x Zenith) to 0.23 (72/100, Arina x Iena).

In vitro screening of F_3 zygotic embryos

The average germination rate of F_3 embryos from all 16 populations on MS medium was 96%. VI ranged from 0.00 (0%) to 0.30 (100%) for F_3 embryos derived from a single F_2 plant. Averaged across the different F_3 embryos of one population, VI ranged from 0.04 (Iena x Zenith) to 0.23 (Forno x SN-).

In vitro screening of F_5 mature seeds

The average germination rate of seeds from all 16 F_4-populations on control medium was 97%. VI ranged from 0.05 to 0.30 for single progenies and the mean for each population ranged from 0.23 (69%, SN+ x Boval) to 0.28 (93%, Arina x Iena). F_4 plants derived from different F_3 plants but the same F_2 plant that showed that the level of resistance against glume blotch differed in the in vitro screening for VI up to 0.18.

Parental lines: field –in vitro

The results from the different field trials are described in detail in Wicki et al. (1999). The aim of the present study was to estimate the value of in vitro selection by comparing the in vitro response of the different in vitro screenings to the resistance levels of the corresponding plant material in the different field trials. Comparing the VI of the parental lines on selective media in vitro with the resistance reactions on the ears in the field (Table 2), significant ($P < 0.01$) negative correlations were found between the two traits (Table 3). A genotype showing a high level of resistance in the field (low score) showed a high germination rate in vitro on selective media. This was true for both systems, the culture of zygotic embryos and the culture of mature seeds (Table 3). Between the resistance reaction on the leaves and the VIs, only slightly negative correlations between r= –0.25 (embryo, n.s.) and r =–0.38 (seeds, n.s.) were found. This is in accordance with the low coefficient of correlation between SNEA and SNLF of 0.44 in 1995 and 0.34 in 1996. (Wicki et al. 1999).

Table 3 Coefficients of correlation between the in vitro index (VI) of the parental lines and the resistance reaction on the ear in the field trials in 1995 and 1996 and between VI and the SNAE disease index for the ear described by Keller et al. (1994) for the two batches of *S. nodorum* extract and two different culture systems

Batch	Type	SNEA 95	SNEA 96	SNEA index
Batch 1	Embryos	–0.92**	–0.85**	–0.91**
Batch 2	Embryos	–0.87**	–0.81**	–0.93**
Batch 1	Seeds	–0.92**	–0.86**	–0.89**
Batch 2	Seeds	–0.91**	–0.93**	–0.91**

** $P < 0.01$

F_1 – crosses: field – in vitro

The correlation between the resistance level on the ears in the field (SNEA) and the in vitro screening (VI) was –0.72 ($P < 0.01$), whereas for SNLF and germination on MSN medium it was –0.17 (n.s.). Figure 2 shows the relation between VI and SNEA of the parental lines together with the VI of the F_1 and the SNEA of the F_1. In order to estimate the effect of heterozygosity, the slopes for both the parental lines and the F_1 are shown.

F_3 - progenies: field – in vitro

In contrast to the parental lines and the F_1 progenies, no significant correlation was found between the resistance reaction in the field and the sensitivity of zygotic embryos to a crude extract of *S. nodorum* in vitro in F_3 progenies (data not shown). This is true for progenies from a single population, for all progenies over all populations,

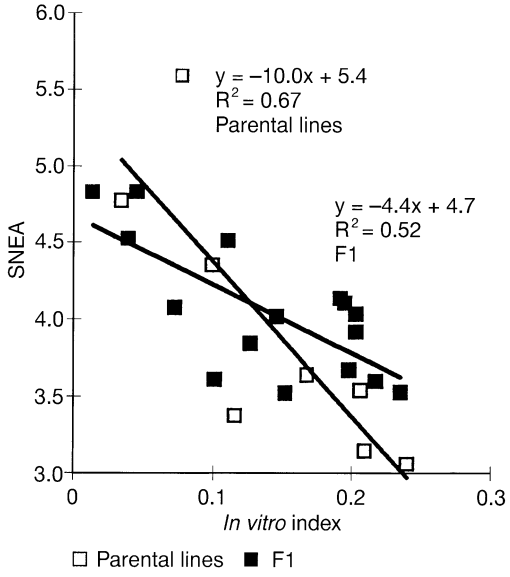

Fig. 2 Relation between resistance reaction on the ear in the field (SNEA) and sensitivity of zygotic embryos to a crude extract (batch 2) of *S. nodorum* in vitro (in vitro index)

and for the mean of the 16 populations. The highest negative correlation was found for the population Iena x Forno (r = –0.29 n.s.).

F_4 - progenies: field – in vitro

The correlation between the recorded SNEA of F_4 headrows in the field and the VI of the in vitro screening over all populations and progenies was r = -0.47 ($P < 0.01$, Fig. 3). The population mean for SNEA was highly correlated with the mean VI (r = -0.89 $P < 0.01$, Fig. 4), indicating that the in vitro test allows one to differentiate between populations for their resistance level on the ear. The correlation was negative for all populations, with the lowest correlation for SN- x Greif (r = –0.11, $P < 0.05$) and the highest correlation for SN+ x Iena (r = - 0.64, $P < 0.01$). Table 4 shows a summary of the regressions for all populations with the corresponding R^2.

Correlation between generations

In this study, a broad data set concerning resistance against *S. nodorum* glume blotch in the field and the in vitro response was obtained over different generations. The question arises, whether it would be possible to predict the level of resistance of the offspring by applying an *in vitro* screening of the parental lines. The correlation between the mean VI of the parental lines and the VI of the F_1 zygotic embryos was r = 0.70 ($P < 0.01$). Between the VI of the parental lines and the SNEA of the F_1 in the field the correlation was r = –0.62 ($P < 0.05$). Correlations were somewhat lower between the mean VI of the parental lines and the mean VI of the

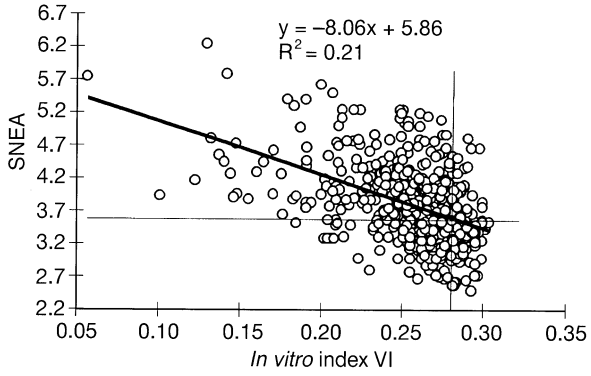

Fig. 3 Regression of the relation between the resistance level on the ear to *S. nodorum* in the field in the generation F_4 and the sensitivty of seeds to metabolites of the pathoge in vitro. Values of all individual from the 16 populations are indicated. Possible selection limits for VI and SNEA are also indicated

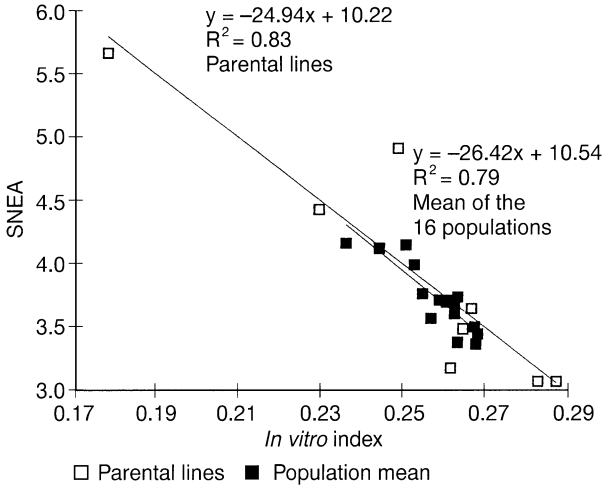

Fig. 4 Regression of the relation between the resistance level on the ear to *S. nodorum* in the field and the sensitivity of seeds to metabolites of the pathogen in vitro. Values of the parental lines and the 16 population means in the F_4 generation are indicated

F_5 seeds (r = 0.59, $P < 0.05$), as well as between the mean VI of the parental lines and the mean SNEA of the F_4 (r = –0.62, $P < 0.05$), indicating that a certain prediction of a population's resistance level is possible when an in vitro screening of the crossing partners is carried out. The correlation between VI of the F_1 and VI of the F_4 was r = 0.60 ($P < 0.05$) and –0.73 ($P < 0.01$) between VI of the F_1 and the mean SNEA of the F_4. These correlations show that there is a clear interaction between the sensitivity to the toxins of the crude extract in vitro and resistance level on the ear in the field in the material screened in the different generations.

Discussion

In this study, we found a high correlation between the different sensitivities of zygotic embryos or seeds of the

parental lines to the toxic metabolites in the crude *S. nodorum* extract and the resistance reaction on the ear. In our study, the in vitro indices were used to determine the level of resistance of the donor plant and not of the individual embryos or seeds. A wheat line showing tolerance to the pathogenic metabolites in vitro is expected to show a high level of resistance in the field. Therefore, it should be possible to select resistant genotypes based on the in vitro screening of a seed sample. If the in vitro index is high, the remaining seed of this line will be planted in the field for further selection, whereas lines with a high susceptibiltity in vitro could be discarded.

We wanted to evaluate if such a detection of resistance at the in vitro level is possible not only between parental lines but also in early generations. Although a large variation in the sensitivity to the pathogen metabolites was observed in the F_3 generation, the variation of the resistance reaction in the field was not in accordance with the in vitro response. Since we found a very high correlation for the parental lines, this was a very astonishing result. Possible explanations could be the different level of homozygosity in the F_3 generation (75%) compared to the parental lines (100%) and that different F_3 genotypes (although derived from the same F_2 plant) were tested in the field and the in vitro screening. If only three resistance loci were to segregate in our populations, this would result in 27 different genotypes in the F_2 and, depending on the degree of heterozygosity, seeds from each F_2 will segregate in the F_3. Since the resistance reaction to *S. nodorum* is a quantitative trait, segregation for several resistance loci is expected in our populations. Ecker et al. (1989) suggested that at least 3–5 genes are involved in the inheritance of resistance to *S. nodorum* on the ear. The probability that a genotype is homozygous for five loci is 0.24 in the F_3 generation, indicating that heterozygosity might still be very important in the in vitro as well as the field trial.

In the in vitro selection experiment, a number of embryos as large as possible had to be used. Therefore, only one ear (about 40 kernels) of a single F_2 plant was left to produce a head-row progeny. If this head row had a higher or lower level of partial resistance than the mean of the ears that were screened in vitro, this would be a sampling error and a high correlation between these two traits over the screened progenies could not be expected. In addition, for the in vitro screening of the parental lines, there were always enough embryos and enough plants available that were in the optimal growth stage for the embryo culture. This was not true for the F_3 embryos used in this system. Due to the large number of entries, it was not possible to harvest all embryos in the optimal stage. Although in the control this had no visible effect, one cannot exclude that older and bigger embryos showed a higher level of tolerance to the extract *in vitro* than younger and smaller ones. The total number of cultured embryos per genotype was smaller than it was for the parental lines or the F_1. Therefore, we have three possible explanations for the lack of a correlation for the F_3 between in vitro and field embryos: The degree of heterozygosity, a sampling effect resulting from segregation, and the reliability of the embryo culture system. Here it has to be noted that many studies dealing with in vitro selection and segregating populations were abandoned at this early stage due to the missing correlation between in vitro and field characteristics in this early generation (for review see Daub 1986).

To study the effect of heterozygosity, F_1 plants were produced and compared with parental lines. Although the F_1s were 100% heterozygous, VI and SNEA showed similar correlations ($r = -0.72$) to the parental lines ($r = -0.82$). This indicates that the sampling effect in the segregating F_3 is more important than the degree of heterozygosity. However, comparing the slopes of the regression between VI and SNEA for the F_1 and the parental lines (Fig. 2), it is obvious that there is an influence of the degree of heterozygosity, because the slope of the parental lines is steeper. Thus, if in early generations the genotypes differ in their degree of heterozygosity for the resistance loci, the overall correlations might be reduced.

In order to evaluate the hypothesis of a sampling effect in the F_3 due to segregation, ten progenies from the F_3 head rows derived from a single F_2 plant were screened for resistance to *S. nodorum* in the field. And, in fact, in the F_4 generation too, a considerable variation in the resistance reactions between the ten progenies all derived from the same F_2 plant was detected. On the other hand, no more obvious segregation within a head row was detected. Since each row was assessed for the resistance reaction to *S. nodorum*, the sensitivity to the pathogen metabolites in vitro should then be better correlated to the resistance reaction of a single head row in the field. In order to prove this, an improved method of in vitro selection had to be found, because it was not possible to harvest such a large number of ears in the correct growth stage for the embryo culture. Moreover, it would not have been possible to culture such a large number of embryos. We tried therefore to use mature seeds for the in vitro test. If the pathogen metabolites were to diffuse through the caryopsis to the embryo, this would have the same effect as the direct contact of an embryo with these metabolites in the growth medium. Actually this was the case since we found a high correlation firstly between the disease indices of the parental lines and the sensitivity to the metabolites in vitro, and secondly between the sensitivity of embryos and the sensitivity of seeds of the parental lines. Therefore, mature F_5 seeds harvested from each F_4 head row were used for the in vitro screening of all 550 progenies across the 16 populations. This system is very easy to handle and allows one to screen a large number of genotypes in a short time, which is a perequisite for a succsessfull in vitro selection method. The correlation of –0.47 between the sensitivity to the pathogen metabolites in vitro and the resistance reaction on the ear of all progenies over all populations (Fig. 3) partly proved the hypothesis that the segregation in the F_3 was too high, and therefore the sampling error too important, to find a significant correlation between in vitro screening and

resistance in the field. Although the correlation for the F_4 is significant due to the large number of entries and is nevertheless high for a quantitative trait, it would not be sufficient for breeders to select for resistance or to detect susceptibility in segregating populations. On the other hand, taking the mean sensitivity to the pathogen metabolites in vitro and the mean resistance level on the ear of each population, a high correlation between these two traits was found. This leads to the conclusion, that this method could be integrated into wheat breeding programs. Populations showing a high susceptibility to the pathogen metabolites in vitro could be discarded. The slopes of the regression between VI and SNEA are similar for all populations (Table 4), indicating that the sensitivity to toxic compounds in vitro of the different populations is genotype independent, which again is a perequisite for a successful application of in vitro selection.

An application of the in vitro method together with the *Septoria* screening within the wheat breeding Program at the Swiss Federal Research Station for Agroecology and Agriculture (FAL-Reckenholz) over several years would provide more reliable data about the usefulness of this method.

A promising implementation for wheat breeding would be the screening of advanced breeding material, or candidate partners, in a crossing program for resistance on the ear. Since they show a high level of homozygosity and we found high correlations for the parental lines between VI and SNEA, detection of highly resistant or highly susceptible germplasm in vitro should be possible. Comparing the VI of the parental lines with the SNEA of the F_1 and F_4, we found exactly the same correlations for both (–0.62). This indicates that it is possible to predict to a certain degree the resistance level of F_1 progenies or the F_4 population in the field when we screen the corresponding parental lines in vitro. These correlations show that there is a clear interaction between the sensitivity to the toxins of the crude extract in vitro and the resistance level on the ear in the field in the material screened in the different generations. On the other hand, in the resistance study of Wicki et al. (1999), crosses with two parental lines susceptible to *S. nodorum* resulted in some progenies in the F_4 showing a high level of resistance. This provides an explanation for why the correlation between these two traits is not higher. In the same study, dominance for susceptibility of the F_1 was found, which again explains the lack of a high correlation between the VI of the parental lines and the SNEA of the F_1. Depending of the future development of wheat hybrids, in vitro screening could also be applied to this material, as we found a relatively high correlation in the F_1 of the 16 crosses. However, the production of hybrids is not the best tool to improve septoria resistance. In most crosses Wicki et al. (1999) found dominance for susceptibility in the F_1. Depending on the success of implementation of the in vitro selection method, it would also be of interest if this system works also for other species, for example triticale where the production of hybrids shows very promising results. The in vitro screening could be applied either to breeding material or to screen the female and male crossing partners, or the F_1 hybrids itself.

Table 4 Slopes of the regression between VI and SNEA of the 16 poplations and the mean of all populations. Values of a, b and R^2

Population	a	b	R^2
1	– 6.72	5.17	0.20
2	– 6.84	5.63	0.20
3	– 7.59	5.77	0.14
4	– 5.23	5.21	0.25
5	– 7.49	5.34	0.14
6	–11.89	6.67	0.41
7	– 3.20	5.15	0.05
8	–10.50	6.20	0.19
9	– 8.76	5.83	0.19
10	– 5.64	5.19	0.11
11	– 4.06	4.65	0.12
12	– 2.80	4.48	0.01
13	– 7.74	5.80	0.38
14	– 4.91	5.44	0.13
15	–10.33	6.56	0.35
16	– 4.52	5.30	0.07
Mean	– 8.06	5.86	0.21

Many studies have been conducted to screen for resistance at the seedling stage. Reactions observed in the greenhouse on seedlings, or in the laboratory on detached leaves, were often in accord with reactions under natural conditions in the field (Wilkinson et al. 1990). Therefore, a combination of seedling screening for leaf resistance and an in vitro screening for ear resistance would be a powerful tool to select germplasm with more complete resistance on both organs. Such a combination would not be destructive, since the seedlings can later grow to maturity and provide seeds for the in vitro screening as well as for further propagation of promising breeding material. However, resistance reactions against *S. nodorum* observed either at the seedling stage or in detached leaves were not always expressed at the adult plant stage under field conditions (;Trottet and Benacef 1989, Nelson and Marshall 1990; Arseniuk et al. 1991).

A complex of problems concerns the pathogen metabolites in the crude extract. The content of these selective acting metabolites varies from batch to batch (Keller et. al. 1994), and the concentration for the best selectivity has to be adjusted for each batch. Moreover, there is no knowledge about the nature of these metabolites. A purification of the extract would lead to a more-defined solution. A fractionation of the extract could result in different selective fractions, which could then further be chemically analysed and characterised. This could lead to information about the mechanisms responsible for the different sensitivities in vitro and under field conditions. In the frame of this study, we fractionated an extract by high-performance liquid chromatography (HPLC) and this resulted in 12 fractions. Among these fractions there were five not belonging to the mellein or septorin families (Prof. Dr. R. Tabacchi, University of Neuchâtel, personal communication). One of the five fractions tended

to show a selective action to wheat embryos originating from the two standard varieties Arina and Forno. But the amounts of these fractions were too small to confirm this. Nevertheless, this approach would be essential to further develop the in vitro selection method and studies in this field have to be continued.

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