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Natural variation for BYDV resistance in maize

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Abstract

With increasing winter temperatures, Barley yellow dwarf virus (BYDV) is expected to become a prominent problem also in maize cultivation. Breeding for resistance is the best alternative to control the disease and break the transmission cycle of the virus. The objectives of our study were to (I) determine phenotypic and genotypic variation in five segregating populations of maize with respect to BYDV tolerance or resistance as well as (II) quantify the influence of BYDV infection on plant performance traits. In 2011, five segregating populations with a total of 445 genotypes were grown at two locations in Germany. Plants were inoculated with BYDV-PAV transmitted by aphids of the species *Rhopalosiphum padi*. We observed considerable genotypic variance for the traits virus concentration as measured by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as well as expression of symptoms. Furthermore, heritabilities were high for the plant performance traits ear height and plant height. Correlation coefficients between all pairs of traits were significantly different from 0 ($P < 0.05$). Genotypes of the inoculated variant were reduced in plant height by 3 cm, ear height by 6 cm, and flowered 3 days earlier compared to genotypes of the non-inoculated variant. The results of our study suggested a high potential for breeding of BVDY resistant / tolerant maize.

Keywords: Luteovirus, *Zea mays*, transmission cycle

Introduction

Barley yellow dwarf (BYD) is the most widespread virus disease of small grain cereals (Plumb, 1983) and was first detected in barley by Oswald and Houston (1951). It is caused by the BYD virus (BYDV), a luteovirus belonging to the family Luteoviridae (Miller et al, 2004) which is phloem-restricted (Lister and Rochow, 1979) and transmitted by different aphid species (Oswald and Houston, 1951).

Rhopalosiphum padi, one of the main cereal aphids, is deemed to be a good vector for BYDV. It has three main flight periods. In autumn, the aphids migrate to autumn-sown cereals and transmit BYDV from maize to winter wheat and winter barley. Until the sexual overwintering, the aphids can distribute the virus in the field. Later in autumn, *R. padi* migrates to its winter host, the bird-cherry tree, for the sexual overwintering cycle. In spring, the virus-free *R. padi* migrates from the winter host to the winter cereals, where the aphids aquire the virus. During the flight in early summer, they transmit the virus to the spring cereals and maize. The most important flight period for the vectors is the alteration of the aphids from the ripening winter and spring cereals to maize and perennial grasses, before in early autumn the aphids colonize the new sown winter cereals (Henry and Dedryver, 1989). The importance of maize as host plant in the epidemiology of BYDV suggests in turn a relationship between the BYDV occurrence in maize and the infection rate of winter cereals (Plumb, 1983). With climate change, winters are becoming milder in temperate climate zones which enables aphids to overwinter in cereal crops (Irwin, 1990). This leads to a continuous spread of the virus during autumn and winter in the field and to an earlier presence of larger aphid populations in spring time. In addition, high temperatures in spring lead to an early invasion of the vectors from the winter cereals to maize, and therefore to an early attack of maize plants (Harrington et al, 2007). As earlier studies revealed that crop plants are especially sensitive to BYDV infection in early developmental stages (Haack et al, 1999), this suggests an increasing impact of BYD on all cereals and especially maize in the future.

The symptoms detected in BYDV infected maize are red bands at the edge of the leaves and interveinal yellowing of leaves (Loi et al, 2004). Furthermore, the results of earlier studies suggested that BYDV infection might lead in maize to a reduction of plant height (Beuve et al, 1999; Loi et al, 2004), total plant fresh weight (Panayotou, 1977), and grain yield (Beuve et al, 1999; Pearson and Robb, 1984). However, a systematic analysis of the influence of BYDV infection on plant performance trait on various genetic material is still missing.

The aphids transmitting BYDV can be controlled by spraying insecticides. However, this is a cost and labor intense approach and harmful for the environment. Therefore, breeding for BYDV resistance is the best alternative to control the disease and avoid reduction of plant performance caused by the virus

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(Ordon et al, 2004).

Not all infected genotypes show symptoms (Grüntzig et al, 1997), and, thus a distinction can be made between tolerant genotypes which are symptomless or which show only week symptoms but allow BYDV to replicate, and resistant genotypes which do not show symptoms and in which the virus can not replicate or only to a low extent (Osler et al, 1985). Only with resistant maize it is possible to break the epidemiological cycle of BYDV and also improve the BYDV situation in other cereals. The prerequisite for improving the BYDV resistance by breeding is genetic variation in the trait of interest. Loi et al (1986) described Ky226 as tolerant, because it did not show symptoms. In contrast, W64A was described to be highly susceptible. In experiments of Grüntzig and Fuchs (2000), FAP1360A showed a very low infection rate and low extinction values in ELISA tests and also D408 was resistant. Furthermore it was shown, that FAP1360A is resistant against Sugarcane mosaic virus (SCMV) (Dußle et al, 2000). In our experiment, these parental inbreds were used for the creation of five segregating populations.

The objectives of this study were to i) determine phenotypic and genotypic variation in five segregating populations of maize with respect to BYDV tolerance and resistance as well as ii) quantify the influence of BYDV infection on the plant performance traits plant height, ear height, and flowering time.

Materials and Methods

Five segregating populations with a total of 445 entries derived from biparental crosses of five inbred lines were examined in our study (Figure 1). The field experiments were carried out at Borken and Wadersloh (North Rhine-Westphalia, Germany) in 2011. Each population was planted separately in a single trial to reduce neighbor effects. The experimental design of each trial was an α lattice design, where the five parental inbreds served as checks. With a sowing machine, 15 untreated seeds of each genotype were sown in a single row plot with a length of 2.3 m. The distance between neighboring plots was 75 cm.

Viruliferous aphids of the species *Rhopalosiphum padi* were reared for 3 weeks in a growth chamber at 20°C on *Triticum aestivum* cultivar "Tuareg" infected with BYDV-PAV. In the field experiment, at each location there was one replicate served as non-inoculated control whereas as one replicate was inoculated with BYDV two weeks after sowing. For the inoculation, a piece of wheat leaf with about 5-10 aphids was placed in the leaf axil of each plant. To protect the seeds from birds, to prevent escape of the aphids and to avoid aphid attack on plants of the non-inoculated variant the plants of the inoculated variant as well the plants of the non-inoculated variant were covered separately with fleece Climatex (17g qm^{-1}). One week after inoculation, the fleece was removed and both variants were sprayed with the insecticide "Biscaya" (Bayer Crop Science Monheim, Germany, 300 ml ha^{-1}).

Six weeks after inoculation, leaf symptoms caused by BYDV like leaf reddening in different intensity (RE) (Beuve et al, 1999; Grüntzig et al, 1997; Osler et al, 1985; Stoner, 1977) as well as yellow leaf stripes (YS) (Loi et al, 2004) were scored on a scale from 1 to 9 (1 = no symptoms, 9 = highest symptom expression). The number of days after sowing until which 50% of the plants in one plot were flowering (FT) was recorded. After flowering, ear height (EH) was measured from the soil line to the node of the upper ear and plant height (PH) was measured from the soil line to the top of the tassel. To analyze BYDV concentration, six weeks after inoculation plant material from the sixth leaf was collected separately for each plant of each plot and each leaf was analyzed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) according to Clark and Adams (1977) using the in-house polyclonal antisera (BYDV-PAV) from the Julius Kühn-Institute (JKI). Preparation of the BYDV-PAV antisera was carried out on the purified BYDV-PAV 1 ASL isolate originating from a field near Aschersleben according to Proll et al (1984). Virus extinction (EX) was estimated at 405 nm on a microtitre plate reader (Opsys MR, Egelsbach, Germany) 1 h after the incubation of the enzyme substrate. For some statistical analyses it was necessary to assign plants to classes based on their EX value. Plants with an EX < 0.5 were classified as resistant. The percentage of plants of one plot with EX ≥ 0.5 was designated as infection rate (IR).

Statistical analyses

We used the following mixed model to analyse the data of all traits collected for the checks across the five trials at both locations separately for the inoculated as well as the non-inoculated variant:

$$Y_{ijk} = \mu + L_i + T_j + C_k + e_{ijk} \quad (1)$$

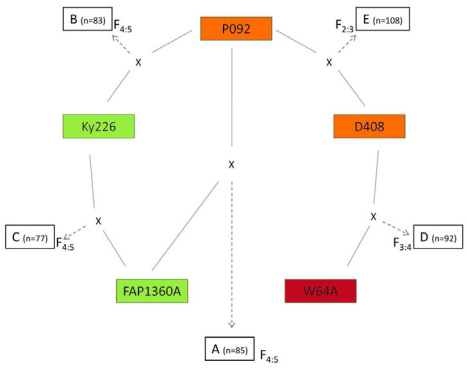

Figure 1- Mating design used to create the five segregating populations A-E from the parental inbreds. Red colored boxes mark parental inbreds with susceptibility to Barley yellow dwarf (BYD) disease, orange color stands for tolerance against BYD disease, and green color stands for resistance.

Table 1 - Genotypic variances and heritabilities of the traits assessed in our study.

Trait	Abbreviation	Segregating population A	B	C	D	E
Inoculated variant						
Yellow stripes	YS	0.27-0.42	0.49-0.49	0.48-0.62	0.31-0.45	0.09-0.28
Red edges	RE	0.60-0.66	0.48-0.78	0.15-0.67	7.37-0.89	0.18-0.77
Virus extinction	EX	0.08-0.68	0.04-0.71	0.03-0.59	0.03-0.56	0.06-0.69
Infection rate	IR	475.71-0.72	484.29-0.75	259.64-0.71	247.58-0.62	271.90-0.62
Flowering time	FT	27.38-0.68	14.36-0.69	20.73-0.84	4.41-0.40	18.92-0.58
Plant height	PH	730.51-0.92	551.27-0.88	906.41-0.94	999.82-0.94	926.53-0.81
Ear height	EH	224.38-0.88	200.48-0.76	344.97-0.91	161.23-0.83	32.48-0.34
Non-inoculated variant						
Yellow stripes	YS	0.00-0.01	0.18-0.52	0.11-0.43	0.05-0.32	0.00-0.00
Red edges	RE	0.00-0.00	0.00-0.00	0.00-0.00	0.00-0.00	0.00-0.00
Virus extinction	EX					
Infection rate	IR					
Flowering time	FT	28.85-0.62	12.68-0.65	14.50-0.72	9.35-0.55	17.84-0.53
Plant height	PH	683.69-0.94	669.10-0.87	780.08-0.93	893.46-0.93	277.01-0.80
Ear height	EH	190.44-0.85	289.05-0.79	302.22-0.90	151.45-0.81	93.43-0.73

Data are represented as "variance-heritability"; traits IR and EX have not been evaluated in the non-inoculated variant.

where Y_{ijk} was the phenotypic observation for the k^{th} check at the i^{th} location of the j^{th} trial, μ the general mean, L_i the effect of the i^{th} location, T_j the effect of the j^{th} trial, C_k the effect of the k^{th} check, and e_{ijk} the residual. All effects were regarded as fixed. The trial effect was subtracted from the raw data of all entries of the corresponding trial to correct for the differences among the different trials. Data of all entries were then analyzed according to the following linear mixed model:

$$Y_{ijlmn} - T_j = \mu + L_i + D_l(TG)_{jm} + (LB)_{in} + e_{ijlmn} \quad (2)$$

where Y_{ijlmn} was the phenotypic observation for the m^{th} entry at the i^{th} location in the n^{th} incomplete block of the j^{th} trial. For the trait EX, Y_{ijlmn} was the mean of the EX values from all plants of one plot. $D_l(TG)_{jm}$ was the interaction effect of the m^{th} genotype, the effect of the j^{th} trial and $(D_{1\text{-}5})_l$ which was an indicator variable with $D_l = 0$ for checks and $D_l = 1$-5 for the entry of the 1st - 5th trial. The latter enabled the calculation of specific genotypic σ^2_g and error σ^2_e variances for the entries of each trial, i.e. the individual segregating populations. $(LB)_{in}$ was the interaction effect of the of the i^{th} location and the n^{th} block, and e_{ijlmn} the residual. L was regarded as fixed, whereas DTG and LB were regarded as random. Heritability (H^2) was calculated for each segregating population based on the formula

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{n}} \quad (3)$$

where n was the number of locations. For all entries, adjusted entry means were calculated as:

$$M_m = \hat{\mu} + \hat{G}_m \quad (4)$$

where μ was the estimate for the intercept and G_m the estimate of the genetic effect of the m^{th} entry calculated based on formula 2. All mixed model analyses were performed using the software ASReml (Gilmour et al, 2006).

For each segregating population, the correlation coefficients among all pairs of traits were calculated based on the adjusted entry means. For both classes, namely genotypes with EX ≥ 0.5 as well as genotypes with EX < 0.5, differences between the inoculated and not inoculated variants with respect to the plant performance traits, PH, EH and FT were examined for their statistical significance. If not stated differently all analyses were performed with statistical software R (R Core Development Team, 2011).

Results

The residuals of all traits as well as the traits EX, IR, FT, PH and EH (Figure 2) were normally distributed. In the inoculated variant, we observed for the plant performance traits EH and PH an average H^2 between 0.74 and 0.90 (Table 1). H^2 for FT was lower and varied considerably with values between 0.40 and 0.84 among the five populations. For RE and EX, we observed with 0.75 and 0.65 high H^2 values. These were lower for YS. H^2 for IR was high with values between 0.62 to 0.75 in all populations. The H^2 trends observed for the performance traits in the non- inoculated variants were similar to that of the inoculated variants. In contrast, in the non-inoculated variants, only half as high H^2 values for the symptom YS compared to the inoculated variants and a H^2 value of 0 for the symptom RE were observed. With respect to the examined traits, we observed a considerable variation within the individual segregating populations. Population C showed the lowest genotypic variance for both RE and EX (Table 1). In contrast, population A varied the most for EX with a genotypic variance of 0.08 and population D the most for RE (7.37). The populations and the parental inbreds differed with respect to the

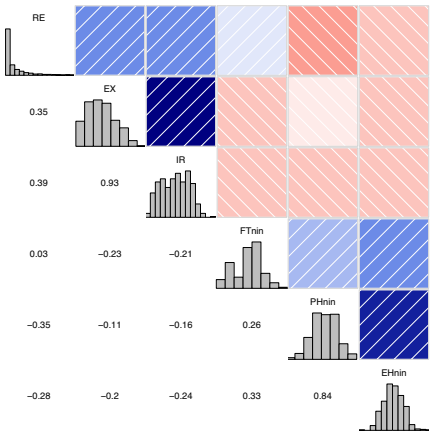

Figure 2 - Correlation of all pairs of traits across the five segregating populations of the inoculated variant for the resistance traits and the non-inoculated variant for the plant performance traits. On the diagonal, the histograms for the traits red edges (RE), extinction rate (EX), infection rate (IR), flowering time in the non-inoculated variant (FTnin), plant height in the non-inoculated variant (PHnin) and ear height in the non-inoculated variant (EHnin) are shown. Blue colors indicate positive correlations where red colors indicate negative correlations. The intensity of the color reflects the strength of the correlation.

mean values of all traits (Table 2). In the parental inbred lines, the highest symptom expression (RE) was found for W64A, whereas P092 showed the highest virus extinction and infection rates, without showing any symptoms. For the parental inbred FAP1360A, symptoms were almost seven scores lower than for W64A. The parental inbreds FAP1360A and Ky226 showed EX values < 0.5. Genotypes in population C showed the lowest RE ratings, the lowest EX values, and the lowest IR. In contrast, in population D the symptom RE was more than two scores higher, EX values were 0.46 higher, and IR was increased of 47% compared to population C.

Genotypes of the inoculated variant with EX ≥ 0.5 were reduced in PH compared to the corresponding non-inoculated variant by an average of 2% across all segregating populations (Table 3). In population E, this difference was statistically significant from 0 ($P < 0.05$). For the genotypes with EX < 0.5, the reduction between inoculated and their corresponding non-inoculated variant was half as high compared to the genotypes with EX ≥ 0.5. EH was, except in population A, significantly reduced by an average of 9% in the genotypes with EX ≥ 0.5 compared to the corresponding non-inoculated variant. In contrast, for the genotypes with EX < 0.5, the reduction was only 7%. Genotypes of the inoculated variant flowered in all segregating populations, except population C and E, significantly ($P < 0.05$) earlier than the non-inoculated variant. The difference between the inoculated and the corresponding non-inoculated variant was

Table 2 - Adjusted entry means of the traits red edges (RE), extinction value (EX) and infection rate (IR) in the inoculated variant.

Entry type	No of lines	Traits RE	EX	IR
Segregating populations				
A (P092 x FAP1360A)	85	1.50	0.64	52.84
B (P092 x Ky226)	83	1.52	0.46	40.94
C (Ky226 x FAP1360A)	77	1.29	0.28	20.56
D (D408 x W64A)	92	3.65	0.74	67.60
E (D408 x P092)	108	1.30	0.70	58.69
Parental inbreds				
Ky226 (resistant)		1.34	0.23	14.36
W64A (susceptible)		7.56	0.74	63.11
FAP1360A (resistant)		0.90	0.43	37.03
P092 (tolerant)		0.91	1.02	82.75
D408 (tolerant)		0.93	0.82	58.38

stronger for genotypes with EX ≥ 0.5 than for genotypes with EX < 0.5. For the parental inbreds, the differences between the two variants followed the same trend as the average across all populations but was for non of the plant performance traits significantly different from 0 ($P < 0.05$). The parental inbred Ky226 showed EX values < 0.5. For this parental inbred all traits were not reduced in the inoculated variant. In the other parental inbred with EX values < 0.5 (FAP1360A), PH and EH were reduced by 7 cm in the inoculated variant, whereas FT was not reduced. The parental inbreds with EX ≥ 0.5 were strongly reduced in PH and EH in the inoculated variant compared to the non-inoculated variant. The strongest reduction was observed for the parental inbred W64A with 21 cm in PH and 10 cm in EH. Flowering time was only 1 day earlier for this parental inbred. Also the parental inbreds P094 and D408 showed reduced PH and EH in the inoculated variant compared to the non-inoculated variant but no earlier flowering. P092 flowered even two days later in the inoculated variant. The correlation between EX and the difference of FT between the inoculated and non-inoculated variant was significantly ($P < 0.05$) negative, whereas EX correlated significantly ($P < 0.05$) positive with the differences between the inoculated and non-inoculated variants for the traits PH and EH (Figure 3).

All pairs of traits showed significant ($P < 0.05$) correlation coefficients, except the correlation between RE and FT (Figure 2). All plant performance traits assessed in the non-inoculated variant correlated positively with each other. Furthermore, the resistance traits RE, EX, and IR were positively correlated among each other but negatively correlated with plant performance traits collected in the non-inoculated variant. The correlation coefficient between symptom occurrence RE and virus extinction EX was with 0.35 significantly different from 0 ($P <0.05$). In the single populations, the observed correlations differed from the correlations observed across all populations (Supplementary Figures 1 - 5).

Table 3 - Comparison of plant height (PH), ear height (EH), and flowering time (FT) between genotypes of the inoculated and non-inoculated variant in the five segregating populations and parental inbreds depending on the observed virus extinction values (EX).

Mean of population	No of lines	Genotypes of the inoculated variant with EX < 0.5	Genotypes of the non-inoculated variant[a]	*P* value	Genotypes of the inoculated variant with EX ≥ 0.5	Genotypes of the non-inoculated variant[b]	*P* value
PH							
A (P092 x FAP1360A)	85	189.46	183.94	0.41	197.16	194.09	0.56
B (P092 x Ky226)	83	195.39	197.86	0.66	192.15	194.16	0.73
C (Ky226 x FAP1360A)	77	196.25	201.11	0.35	196.17	199.42	0.77
D (D408 x W64A)	92	157.40	163.50	0.36	155.48	162.80	0.05
E (D408 x P092)	108	179.19	182.98	0.49	186.66	193.40	0.02
All populations	445	189.30	191.64	0.42	180.27	184.41	0.09
Ky226 (resistant)	1	165.23	164.94				
W64A (susceptible)	1				129.82	150.92	
FAP1360A (resistant)	1	159.21	166.03				
P092 (tolerant)	1				178.03	181.93	
D408 (tolerant)	1				147.61	156.05	
All parental inbreds	5	162.22	165.48	0.47	151.82	162.96	0.55
EH							
A (P092 x FAP1360A)	85	75.63	75.80	0.96	82.53	82.10	0.89
B (P092 x Ky226)	83	82.33	89.11	0.06	79.48	88.77	0.03
C (Ky226 x FAP1360A)	77	88.07	94.98	0.02	85.69	94.20	0.03
D (D408 x W64A)	92	60.71	70.10	5.6e^{-03}	60.15	68.00	3.3e^{-04}
E (D408 x P092)	108	68.71	75.18	0.04	71.73	81.79	5.3e^{-09}
All populations	445	79.37	85.12	1.8e^{-03}	71.96	79.05	8.8e^{-07}
Ky226 (resistant)	1	78.39	77.61				
W64A (susceptible)	1				48.64	58.57	
FAP1360A (resistant)	1	67.38	75.53				
P092 (tolerant)	1				67.49	73.85	
D408 (tolerant)	1				55.87	63.04	
All parental inbreds	5	72.89	76.57	0.62	57.33	65.15	0.34
FT							
A (P092 x FAP1360A)	85	82.22	88.26	9.8e^{-05}	83.12	88.99	1.0e^{-05}
B (P092 x Ky226)	83	96.97	100.66	1.4e^{-06}	98.06	102.67	8.6e^{-04}
C (Ky226 x FAP1360A)	77	93.29	97.46	2.2e^{-08}	90.59	93.50	0.37
D (D408 x W64A)	92	89.28	91.36	0.13	89.77	92.78	2.7e^{-06}
E (D408 x P092)	108	86.31	85.49	0.57	87.10	87.06	0.96
All populations	445	90.86	94.30	3.4e^{-06}	88.78	91.64	1.1e^{-05}
Ky226 (resistant)	1	108.27	107.18				
W64A (susceptible)	1				95.80	96.60	
FAP1360A (resistant)	1	79.92	79.22				
P092 (tolerant)	1				94.06	92.82	
D408 (tolerant)	1				87.22	87.66	
All parental inbreds	5	94.10	93.20	0.97	92.36	92.36	1.00

[a] genotypes which showed in the inoculated variant an EX <0.5; [b]genotypes which showed in the inoculated variant an EX ≥0.5

Discussion

The problem of BYDV in maize and its effect on plant performance

An increasing problem of BYD in Germany is expected due to global warming (Habekuß et al, 2009; Tiedemann and Ulber, 2008). In mild winters, the aphids, transmitting BYDV survive in asexual populations and can infect maize plants earlier in very susceptible plant stages. In order to evaluate the effect of BYDV infection on maize during these stages on plant performance traits, we compared a inoculated and a non-inoculated variant with regard to the traits FT, EH, and PH. All traits were reduced in BYDV infected maize compared to the non-inoculated variant. However, the reduction over all populations was only 3 cm for PH, 6 cm for EH and 3 days for FT (Table 3) and in most of the populations not significant ($P < 0.05$). The low reduction of PH confirms the results of Grüntzig et al (1997) where no or little reduction of PH was observed.

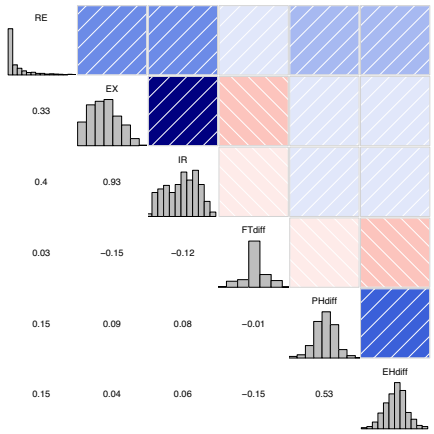

Figure 3 - Correlation of the traits red edges (RE), extinction rate (EX), and infection rate (IR) of the inoculated variant with the differences between genotypes of the non-inoculated variant and the inoculated variant of the plant performance traits flowering time (FT), plant height (PH) and ear height (EH). Blue colors indicate positive correlations where red colors indicate negative correlations. The intensity of the color reflects the strength of the correlation.

Considering the class of genotypes with EX ≥ 0.5 in comparison to the class of genotypes with EX < 0.5, the reduction of PH and EH between the inoculated and non-inoculated variant was stronger for the the former than the latter. This result is in accordance with the correlation analysis (Figure 3), which revealed that the higher the EX and IR in the inoculated variant are, the higher was the reduction in PH and EH. This finding indicates that susceptible genotypes are stronger reduced in the plant performance traits PH and EH than resistant genotypes. This is clearly shown in the parental inbreds, where the susceptible parental inbred W64A is much stronger reduced for the traits PH and EH than the resistant parental inbreds (Table 3).

For the trait FT, we observed a negative correlation of EX of the inoculated variant and the difference in flowering time between the non-inoculated variant and the inoculated variant. That means, resistant genotypes showing low EX values have stronger differences in FT. This finding might be due to the ability of resistant genotypes to flower early in environments with high BYD pressure (Takeno, 2012).

The results of our study suggested that compared to the strong negative effects of BYDV reported for small grain cereals (Baltenberger et al, 1987) the damage in maize is low. A possible explanation for our finding might be that maize is not the main host of BYDV. Therefore, from this point of view BYD is of limited economic importance for maize. But as maize serves as an important alternate host and is therefore of big importance in the transmission cycle of BYDV, breeding of BYDV resistant maize is important to break the epidemiological cycle. This would reduce the BYDV problem in other cereals indirectly.

Environmental influence on symptom occurrence and success for infection of maize

We observed a high H^2 for RE in the inoculated variant. Our finding indicates a strong influence of the genotype on this symptom (Table 1). Together with the fact that no red edges were observed for genotypes of the non-inoculated variant, we considered reddening of the leaf edges as a very characteristic symptom for BYDV infection. This was in accordance with earlier studies (Beuve et al, 1999; Grüntzig et al, 1997; Osler et al, 1985; Stoner, 1977).

In our study, the symptom YS was less heritable than RE. Despite lower YS appearance in the non-inoculated variant, YS were still present in the non-inoculated variant. Our observation suggests that YS is not characteristic for BYDV infection. An explanation for our observation might be that chlorosis or yellowing at the leaf veins are caused not only by BYDV but also by other environmental factors such as nutrient deficiencies/excess (Marschner, 1995). This result is in contrast to results of Loi et al (2004) and Panayotou (1977).

An environmental influence was also observed on the mean IR. The IR was in average across all genotypes and both locations 48%. In Borken, the mean IR was 64% whereas in Wadersloh a mean IR of 30% was observed (results not shown). One reason for the low infection rate in Wadersloh was probably the hot and dry weather in May 2011. Due to these conditions, not all plants of one plot were germinated, when the inoculation was carried out. Therefore, late germinated plants were not infected with BYDV. Furthermore, temperatures of 30°C have a negative influence on the survival rate of *R. padi* (Beuve et al, 1999; Dean, 1974) which can lead to low IR (Grüntzig et al, 1997). Therefore, the high temperatures after inoculation could have led to the low IR in Wadersloh. Nevertheless, we observed a high H^2 for the trait EX and IR, which indicates that the improvement of BYDV resistance by breeding is even under such conditions possible.

Tolerance versus resistance against BYDV

We observed that the symptom RE is a good indicator for a BYDV infection. This is confirmed by the significant positive correlation of RE and EX (Figure 2), i.e. genotypes with strong symptoms have a high virus concentration and are therefore classified as susceptible. Such genotypes are located in the 1st quadrant of Figure 4. Nevertheless, the correlation was by far not perfect. This can be explained thereby that BYDV resistance has to be distinguished from tolerance. Tolerant genotypes are defined as genotypes without any BYDV symptoms but high EX values. The genotypes with low scores for RE but EX values ≥ 0.5 are located in the 2nd quadrant of Figure 4 and are classified as tolerant. Such symptomless carrier of BYDV in maize, already described by Osler et al (1985), are not able to break the transmission

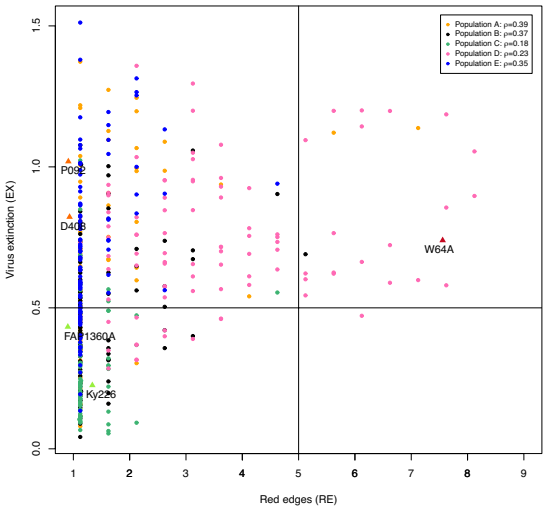

Figure 4 - Covariation of the traits red edges (RE) and virus extinction (EX) in the five segregating populations. The horizontal line at EX = 0.5 is the threshold between resistant and susceptible genotypes and the vertical line defines low symptoms at the left hand side and strong symptoms at the right hand side. Red colored symbols at the parental inbreds mark susceptibility to barley yellow dwarf (BYD) disease, orange colored parents showed resistance and green color stands for resistance.

cycle of BYDV.

The most interesting genotypes for maize breeding are the genotypes without symptoms and EX < 0.5. These genotypes, shown in the 3rd quadrant of Figure 4, are classified as resistant. The resistance mechanism is not known so far. However, the results suggest that on such genotypes the ability of BYDV to replicate is strongly reduced leading to low EX values. Such genotypes are able to break the transmission cycle of BYDV. DAS-ELISA enables the differentiation between tolerant and resistant genotypes. This procedure however is very laborious and not possible to screen in high throughput systems. Therefore, the identification of molecular markers diagnosed for this trait would allow a fast selection for resistant genotypes.

Variation within and between the five segregating populations of maize with regard to BYDV resistance

The mean EX differed among the five segregating populations (Table 2). The most resistant population was population C which was derived from the cross between the two most resistant parental inbred lines Ky226 and FAP1360A. The resistance of these two parental inbreds against BYDV is in accordance with results of Loi et al (1986) and Grüntzig and Fuchs (2000). Furthermore, FAP1360A was resistant against SCMV (Dußle et al, 2000) which leads to the consideration that possibly there are same resistance mechanisms involved in the resistance to SCMV and BYDV. Some genotypes in population B also show low EX values because one of the parental inbreds is the resistant parental inbred line Ky226. Because all lines belong to the Dent pool, the resistant lines could be used in hybrid breeding in the heterotic pool Dent to improve the BYDV resistance in hybrids. Population A, which has been derived from the crossing with the resistant parental inbred FAP1360A showed higher EX values because this line was *per se* less resistant than Ky226. The mean of population E reached a EX value which was almost as high as the mean of the most susceptible population D. This observation can be explained by the fact that the parental inbreds P092, D408, and W64A showed high mean values for EX. The parental inbred D408 showed a good resistance in the experiments of Grüntzig and Fuchs (2000) but in our experiments the EX values were the second highest. The strong susceptibility of the parental inbred W64A observed in our study was also described by Loi et al (1986). The parental lines for the crossings of the segregating populations were chosen based on the information of the literature (Grüntzig and Fuchs, 2000; Loi et al, 1986). This information could be confirmed in our study except the line D408, which in fact did not show symptoms but had hight EX values. That is the explanation, why we had no crossing between resistant and susceptible parental lines.

We observed a normal distribution of EX in each of the segregating populations (Figure 2, Supplementary Figures 1 - 5). Our observation might be due to a high environmental influence on these traits. However, the moderate to high heritabilities observed across the two locations suggested that this might be rather due to a polygenic inheritance of the resistance to BYDV in maize. This finding was in accordance to Grüntzig and Fuchs (2000).

We observed that the stronger the two parental inbreds differed with respect to EX but also RE, the higher was the genetic variance of the corresponding trait in the segregating population (Table 1). This finding suggested the absence of strong epistatic interaction for the inheritance of RE and EX. We observed in all populations transgressive segregation, i.e. single entries which reached a better resistance level than their parental inbreds. This finding could be due to the complementary action of additive alleles that are dispersed between the parental inbreds (Riesberg et al, 1999) with different resistance mechanisms. To check these hypothesis and identify genome regions which are involved in the expression of BYDV resistance to enable marker assisted selection for breeding of BYDV resistant maize, QTL analyses are required.

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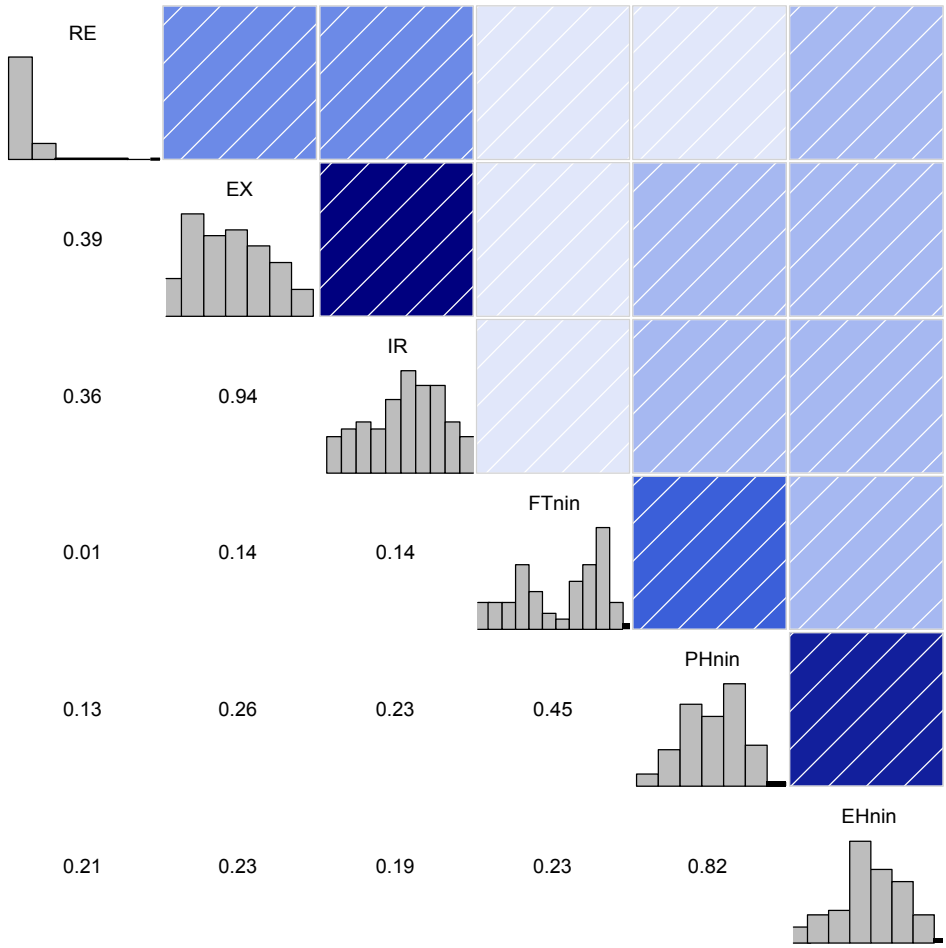
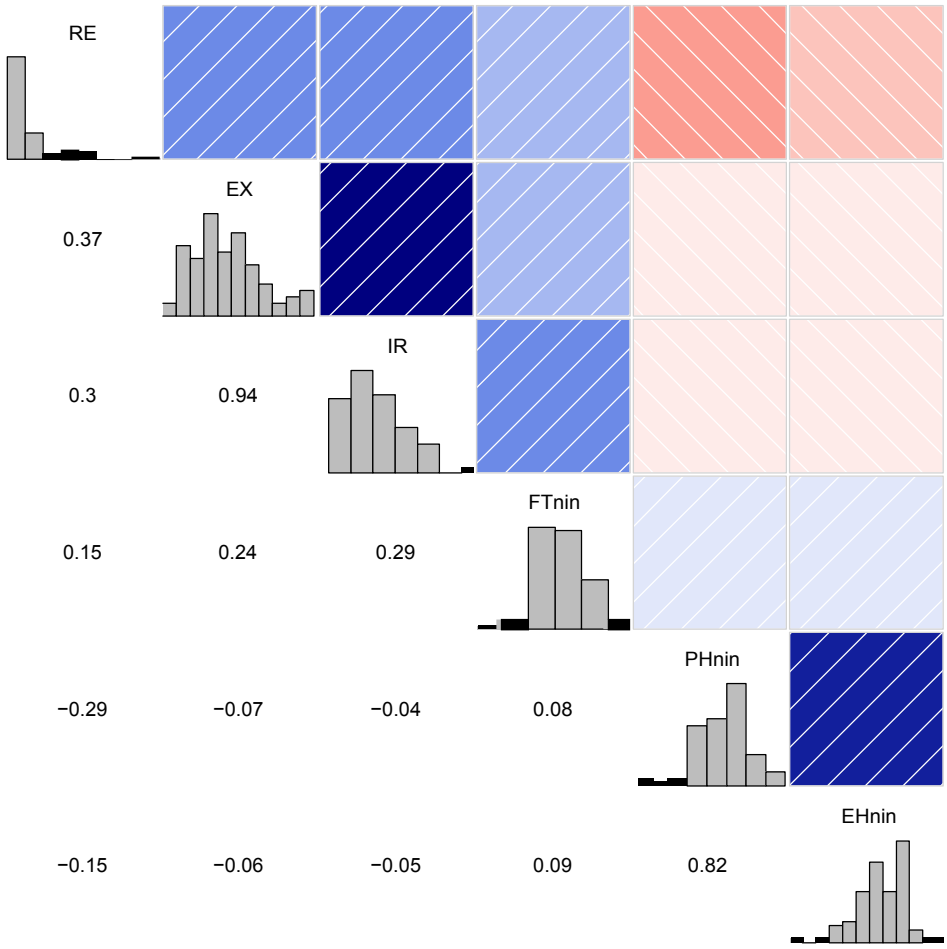
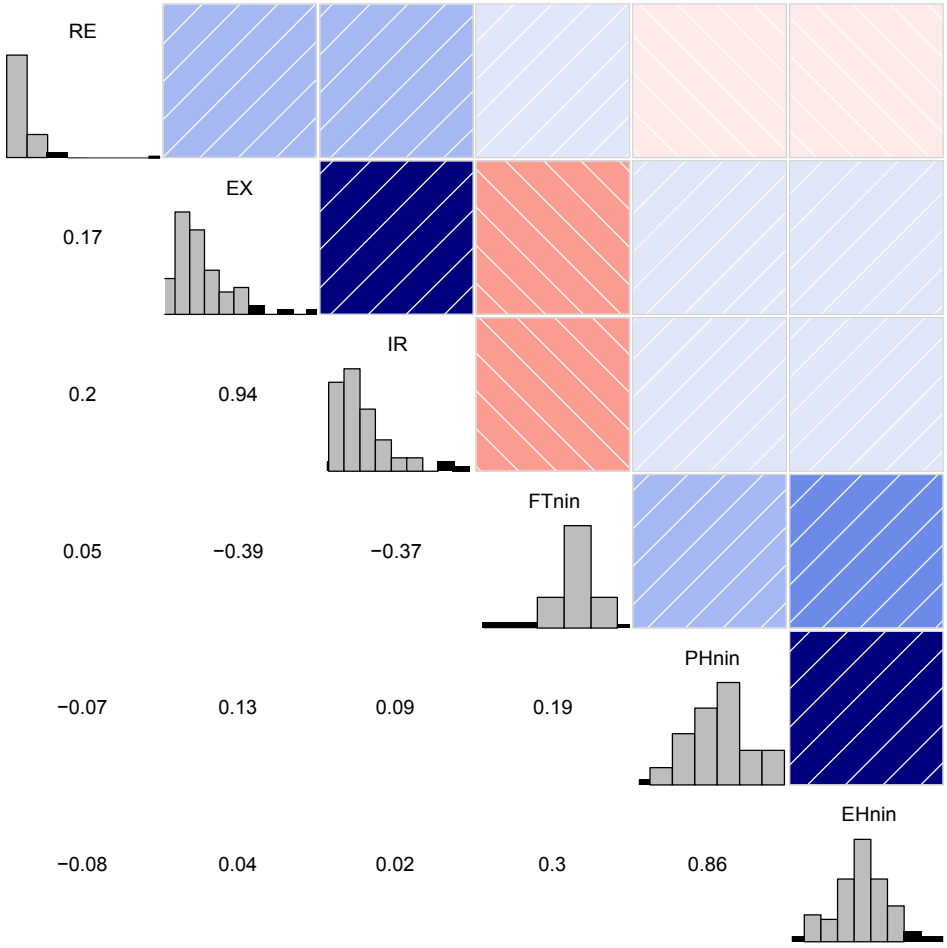


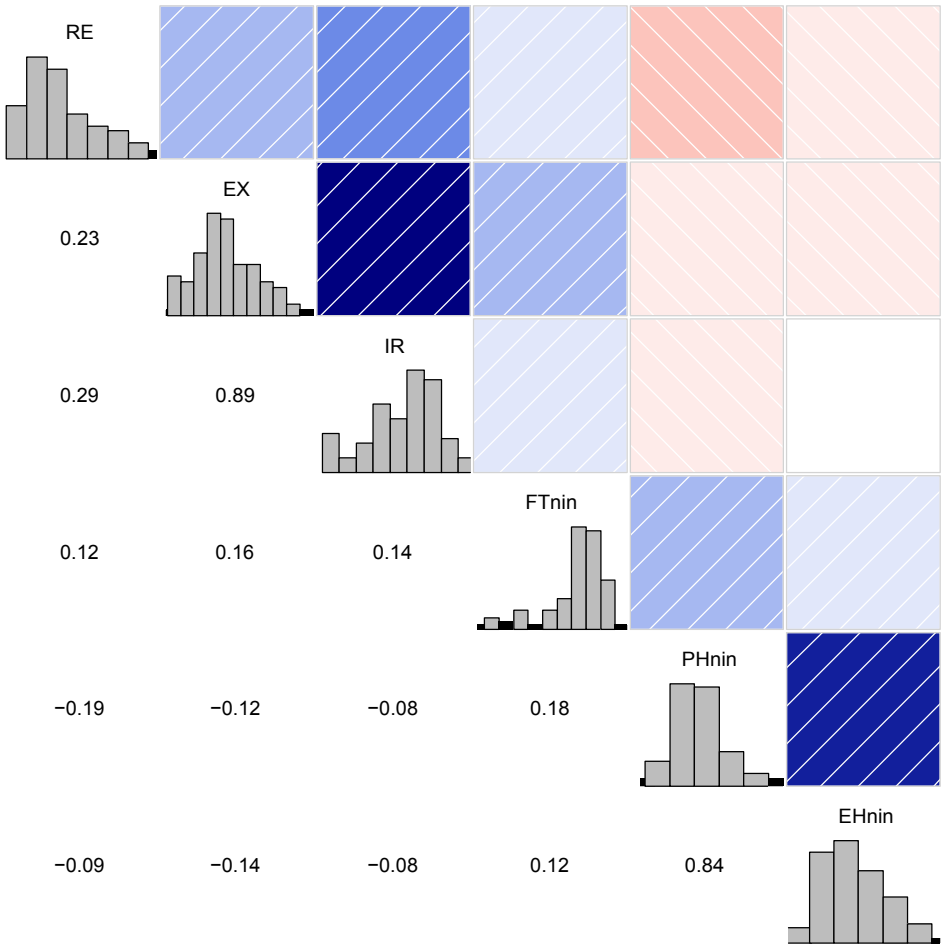
S Figure 1 Correlation of all pairs of traits in Population A (P092 x FAP1360A) of the inoculated variant for the resistance traits and the non-inoculated variant for the plant performance traits. On the diagonal, the histograms for the traits red edges (RE), extinction rate (EX), infection rate (IR), flowering time in the non-inoculated variant (FTnin), plant height in the non-inoculated variant (PHnin) and ear height in the non-inoculated variant (EHnin) are shown. Blue colors indicate positive correlations where red colors indicate negative correlations. The intensity of the color reflects the strength of the correlation.



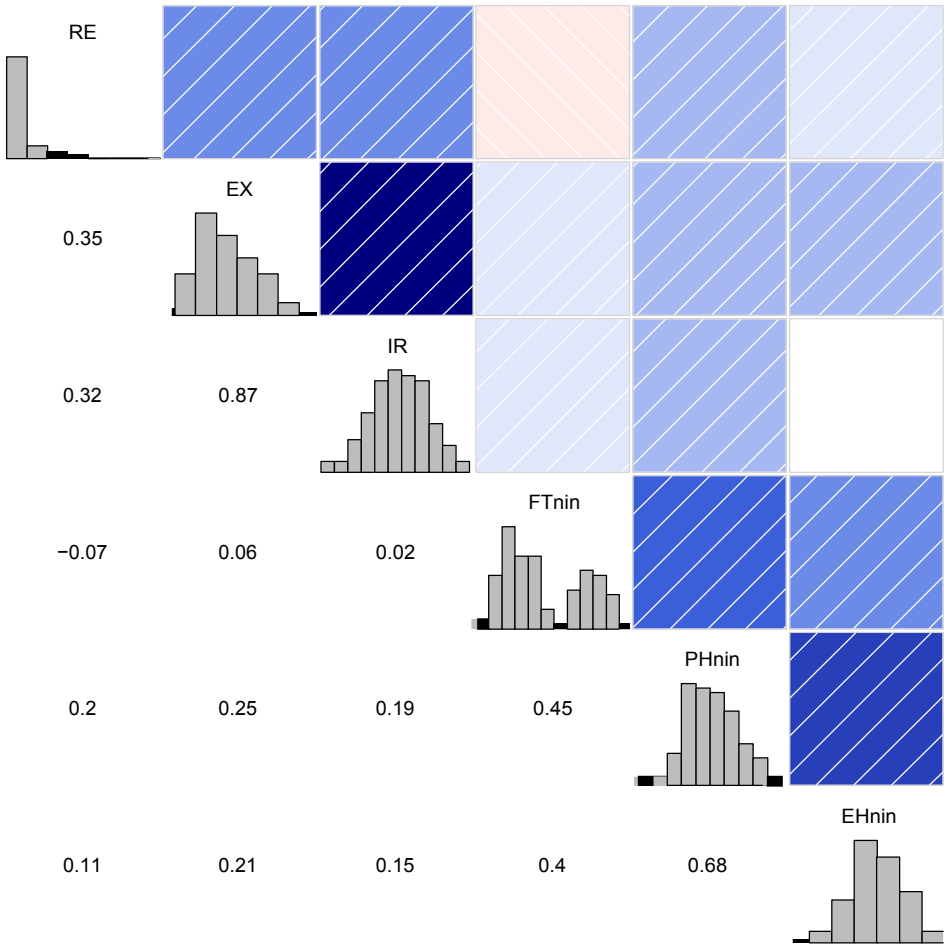
S Figure 2 Correlation of all pairs of traits in Population B (P092 x Ky226) of the inoculated variant for the resistance traits and the non-inoculated variant for the plant performance traits. On the diagonal, the histograms for the traits red edges (RE), extinction rate (EX), infection rate (IR), flowering time in the non-inoculated variant (FTnin), plant height in the non-inoculated variant (PHnin) and ear height in the non-inoculated variant (EHnin) are shown. Blue colors indicate positive correlations where red colors indicate negative correlations. The intensity of the color reflects the strength of the correlation.



S Figure 3 Correlation of all pairs of traits in Population C (Ky226 x FAP1360A) of the inoculated variant for the resistance traits and the non-inoculated variant for the plant performance traits. On the diagonal, the histograms for the traits red edges (RE), extinction rate (EX), infection rate (IR), flowering time in the non-inoculated variant (FTnin), plant height in the non-inoculated variant (PHnin) and ear height in the non-inoculated variant (EHnin) are shown. Blue colors indicate positive correlations where red colors indicate negative correlations. The intensity of the color reflects the strength of the correlation.



S Figure 4 Correlation of all pairs of traits in Population D (D408 x W64A) of the inoculated variant for the resistance traits and the non-inoculated variant for the plant performance traits. On the diagonal, the histograms for the traits red edges (RE), extinction rate (EX), infection rate (IR), flowering time in the non-inoculated variant (FTnin), plant height in the non-inoculated variant (PHnin) and ear height in the non-inoculated variant (EHnin) are shown. Blue colors indicate positive correlations where red colors indicate negative correlations. The intensity of the color reflects the strength of the correlation.



S Figure 5 Correlation of all pairs of traits in Population E (D408 x P092) of the inoculated variant for the resistance traits and the non-inoculated variant for the plant performance traits. On the diagonal, the histograms for the traits red edges (RE), extinction rate (EX), infection rate (IR), flowering time in the non-inoculated variant (FTnin), plant height in the non-inoculated variant (PHnin) and ear height in the non-inoculated variant (EHnin) are shown. Blue colors indicate positive correlations where red colors indicate negative correlations. The intensity of