Social interactions: breeding for diversity and competition between plants

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Abstract

Modern day agriculture relies heavily on the use of external inputs, with detrimental effects on the surrounding environments. Recently, renewed focus is laid on developing more sustainable farming practices. Intercropping is shown to increase total production without the need of external inputs. In an intercropping trial, multiple crops are grown together in a field. Many modern crops are however bred for monocropping, which might not translate to superior performance in intercropping. Breeding for intercropping requires improving the total systems performance, which means selecting on both direct and indirect genetic effects. Estimating these genetic effects, requires experimental designs, where the different genotypes are grown in multiple combinations. Partial diallel designs are a type of incomplete designs that allow accurate estimation of genetic effects, under limited field capacity. In this study we will investigate how variation in genetic effects, design parameters, and field disturbances influence the estimation accuracy, broad sense heritability, and genetic gain of a bicrop strip intercropping trial following a partial diallel design. The broad sense heritability was estimated with little to no bias for all levels of simulated genetic effects, under all levels of environmental disturbance. Minor interaction between number of genotypes and bias in broad sense heritability was observed. The precision of the genotypic estimates increased, and their bias decreased when larger genetic effects were modelled. Raising the environmental disturbances reduced the precision of the estimates. Increasing the number of genotypic replicates increased the precision of the estimates. Increasing the number of trialled genotypes resulted in less varied estimates of the realized genetic gain, while increasing the plot level disturbance decreased the realized genetic gain.

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Introduction

The art of plant breeding is thought to date back to 10.000 BC and coincide with the formation of the first settlements in what is currently west Turkey (Schlegel, 2017). These early settlers started collecting and multiplying seeds of wheat that had a firmer spike, allowing easier harvesting and threshing. During the following millennia, 'agricultural' knowledge and crops migrated cross the globe and cultivation and improvement of plants became prevalent in multiple locations. Improvements were mainly made by rural communities, but this came to change with the rediscovery of Mendel's work at the end of the 19th century (Reeves & Cassaday, 2002; Simunek, Hoßfeld, & Wissemann, 2011). This discovery is considered to mark the start of science based plant breeding (Duvick, 1996). One of the major achievements of modern scientific plant breeding took place in the 1960s with the development of dwarf type wheat varieties (Trethowan, Reynolds, Ortiz-Monasterio, & Ortiz, 2007). These improved dwarf type varieties were cultivated according to more industrialized agricultural practices, using large amounts of synthetic fertiliser and pesticides (Hedden, 2003). The combination of improved varieties and novel industrial cultivation techniques had a great beneficial effect on the yield and is seen as the start of the Green Revolution. The modern varieties were soon adopted by many farmers and over the following decades, the reliance on external input also further increased (Evenson & Gollin, 2003). The novel developments in agriculture allowed farmers to provide more food to the ever-growing world population, which however also came at a cost. Many local varieties were lost, soils and water supplies were degraded, and overuse of pesticides caused detrimental toxic effect on the surrounding environments (Paroda, 2018). In recent years, much effort is put into developing novel agricultural practices that assure ample yields without negatively affecting the environment. Intercropping is one of these practices regaining attention as it is shown to increase yield stability, improve soil fertility, reduce disease, pest and weed pressure, and increase total production (Roholla Mousavi, 2011).

Intercropping is defined as: 'The cultivation of two or more crops together in the same field for a period of time; the crops may differ by species or cultivar' (Glaze-Corcoran et al., 2020). Many crop varieties are however bred to perform well in a monocrop setting, which might not translate to superior performance in a mixed crop system (Litrico & Violle, 2015). Plants grown in an intercropped system are not only influenced by their own variety or species, but also interact with the differing neighbour. For intercropping, the aim of breeding should be focussed on improving the total systems performance. The main performance of a genotype in a mixture can be described through its general mixing ability (GMA), while the interaction between specific genotypes can be quantified through the

specific mixing ability (SMA) (Pankou, Lithourgidis, Menexes, & Dordas, 2022). In this study, we will focus on the GMA, as we are currently not interested in identifying compatible genotypes in our trial. We will attempt to improve the total systems performance, which requires increasing the GMA (Haug et al., 2021). This translates to a genotype that performs well when grown next to a variety of other genotypes. At the base of GMA lie direct and indirect genetic effects (Haug et al., 2021). Direct genetic effects (DGE) concern the effects that are directly controlled by the individuals own genetics. Indirect genetic effects (IGE) are the effects of an individual's genes that influence its neighbours' performance (Bijma, 2014). The nature of the influence can either have a positive (facilitation) or negative (competition) effect (Haug et al., 2021). In a bicrop system, breeding for total systems productivity would require taking the DGE and IGE of both crops into consideration. In model terms, the total systems productivity of such a bicrop intercropping trial (Y_s) can be explained by the following modified equation (Bourke et al., 2021),

$$Y_s = w_1(\mu_1 + DGE_1 + IGE_2) + w_2(\mu_2 + DGE_2 + IGE_1)$$

w_x represent the importance of each crop in terms of profit,

μ_x is the main contribution of the crop.

Genetic gain resulting in total systems improvement, requires partitioning Y_s into a genotypes DGE and IGE. To enable precise estimation of the DGE and IGE in a bicrop intercropping system with multiple genotypes, care should be taken in developing an appropriate experimental design. The design ought to contain the genotypes in multiple different combinations (Bourke et al., 2021).

Identifying suitable genotypic neighbours requires analysing multiple combinations of genotypes of both crops (Haug et al., 2021). Increasing the number of genotypes in a trial necessitates a larger number of test plots, which may become a limiting factor in a field trial. Incomplete factorial designs are proposed to be used as it enables studying a larger number of genotypic combinations, without increasing the need of extra field capacity (Haug et al., 2021). Partial diallel designs are a type of incomplete designs that allow analysis of a larger number of genotypes in a crop mixture, without resulting in increased field capacity (Forst et al., 2019). In a partial diallel design, only a subset of all genotypic combinations of each crop are present in the field. The number of genotypic replicates dictates how often each genotype is present in the field. Partial diallel designs are proposed to be employed, as they allow accurate estimation of the GMA, while requiring less field resources compared to a full factorial design (Haug et al., 2021).

It has however not been considered how varying crop, environmental, and design factors could influence the accuracy and precision of the estimates in a partial diallel design. Accuracy is defined as the closeness of an estimate to its true value. The accuracy is influenced by random error components and common systematic errors ("ISO 5725-1:1994, n.d.). The random error is typically measured as the standard error (SE) of the estimate, while the systematic error is measured by the bias, the difference between the expected value of the estimate and the true value. In this study we will investigate how variation in genetic effects, environmental disturbances, and number of replicates may affect the estimation accuracy of the DGE and IGE in a bicrop intercropping trial. To determine the accuracy of the estimates, we will evaluate the correlation and regression between the true and estimated IGE and DGE.

As more genotypes can be tested in partial diallel designs relative to full factorial designs, Haug et al. proposed adopting strong selection intensities, which could potentially result in increased genetic gain. Genetic gain (G) is explained by the breeders' equation, which multiplies the additive genetic variability (σ_a), with the selection accuracy (r), and selection intensity (i) (Falconer & Mackay, 1996).

$\Delta G = \sigma_a * r * i$

Since variability in crop and environment may influence the accuracy of the estimates, the selection accuracy could be affected, altering the genetic gain obtained from a trial. We will therefore investigate how variation in genetic effects, environmental disturbance, and number of replicates affects the genetic gain of an intercropping trial. Hereto we will determine the predicted genetic gain after one generation of intercropping.

Besides selection intensity and accuracy of the estimates, genetic variability plays a role in the genetic gain. The amount of variation explained by the genetics of the plant can be explained by the broad sense heritability (H^2) (Piepho & Möhring, 2007). The H^2 is defined as the proportional contribution of genotypic effects (σ_g) to the total phenotypic variation (σ_p).

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

The genotypic variation is composed of the DGE or IGE of a crop, while the phenotypic variation is determined by the sum of genetic components and field disturbances. Accurately partitioning the systems performance (expressed in yield) into its genetic and environmental components is therefore important. We will therefore investigate how the interplay between those aforementioned factors influence the broad sense trial heritability.

Research questions

Our research will centre around the following question: How does variation in genetic effects, design parameters, and field disturbances influence the estimation accuracy, broad sense heritability, and genetic gain of a bicrop strip intercropping trial?

This research question will be broken down into three sub questions:

- How is the estimated DGE and IGE broad sense heritability affected by variation in the magnitude of field disturbances, number of genotypes, and increased DGE and IGE?
- How does enlarging the magnitude of IGE and DGE, affect the bias and precision of the crop and genotypic estimates under increased field disturbance, or with an increased number of genotypic replicates?
- How is the predicted genetic gain after one round of intercropping affected by variation in the magnitude of DGE and IGE, under increased field disturbances, or with an increased number of replicates?

Hypotheses

Broad sense heritability

We expect larger variation in genetic effects to result in less biased broad sense heritability estimates. Increasing the variation in target effects will improve the estimation of their variance components, leading to more accurate heritability estimates.

We hypothesise that large field disturbances to increase the bias of the estimated heritability. Introducing more “noise” to the data will reduce the ability of the model to accurately estimate the variance components, leading to more biased heritability estimates.

Increasing the number of repeated measurements per genotype is expected to increase the bias of the H^2. As the field size is fixed, increasing the number of repeated measurements will mean less genotypes can be trialled in the same field. Reducing the number of levels is indicated to give more biased variance estimates with a larger SE (Mcneish, Stapleton, Mcneish, & Stapleton, 2014; Rameez et al., 2022).

Accuracy of the estimates

We expect that increasing the IGE and DGE will reduce the bias and standard error of the estimates, as enlarging the target effects is found to reduce the bias and SE (Rao & Zaino, 2005).

We hypothesise that increasing the off-target effects (plot level disturbance) will increase the SE of the estimates, as increasing the overall noise in the data, makes estimation of the parameters remaining constant more difficult.

We further expect larger number of repeated measurements (within group replicates) to give more precise estimates of both IGE and DGE. More observations per estimate is expected to improve its estimation.

Realized genetic gain

We hypothesise that increasing the DGE and IGE will result in more accurate predictions of the genetic gain, which coincides with a larger realized genetic gain. This may be expected as larger DGE and IGE are expected to give less biased estimates with smaller SE, resulting in estimates better representing the true values.

We expect less accurate predictions of the genetic gain when the environmental disturbance is raised. Large environmental disturbances are expected to decrease the precision and bias of the estimates. Best estimated genotypes may not coincide with the true best performing genotypes, resulting lower realized genetic gains.

We assume to observe higher realized genetic gains when the number of trialled genotypes is increased. Greater realized genetic gains are expected as the selection intensity remains a fixed percentage, and the sample of genotypes making up the pool for the next generation increases with more trialled genotypes. There may however be a trade-off between number of trialled genotypes and genotypic replicates. More genotypic replicates are expected to improve precision and reduce the bias of the estimated genetic effects, which may be expected to improve the realised genetic gain.

Approach

We will attempt to unravel the interactions between design, crop, and environmental factors through a simulation study. We will simulate various bicrop strip intercropping trials, with varying number of genotypes, with different levels of genetic effects, under increased amounts of environmental disturbance. The simulated trials will be analysed using mixed effects modelling and their outcome evaluated by comparing estimates to the simulated values.

Materials & Methods

The following intercropping simulation study centres around two hypothetical crops, of which the magnitude of the crop parameters may be assumed to represent true values. All simulations and analyses were performed in R Studio version 3.6.3 (R Core Team, 2023).

The intercropping simulation study follows a four-step approach. An empty field is initially generated, after which the different genotypes of the two crops are randomly assigned to a neighbour and randomly assigned to a position in the empty field (Step 1). Base yield data is generated for each genotype and additional neighbouring and field effects are modelled based on their location in the field and added onto the base yield (Step 2). The final yield data is fed into a mixed effects model that predicts the various components that make up the final yield data (Step 3). These estimated effects are compared to their true simulated values and the accuracy of the estimates, trial H^2, and genetic gain is determined (Step 4). Each simulation is repeated 100 times to reduce the chance of false conclusions based on stochasticity of the simulation process.

Step 1:

Field layout and crop allocation

All intercropping trials were simulated to be performed on a field taking the same dimensions and layout as the experimental field located at the Droevendaal Experimental Farm (51.992° N, 5.661° E). The field consisted of 240 plots, divided over 15 rows and 16 strips. The strips will have two crops that interact; the rows are perpendicular to that, and no interactions cross rows are modelled. Each plot was further divided into two subplots, giving a total of 480 subplots. Genotypes of the two crops were assigned to subplots in the following restricted random fashion. Genotypes were hereto first randomly assigned to a genotype of the other crop, following a partial diallel design, giving pairs of neighbouring genotypes. Pairs of neighbouring genotypes were then randomly assigned to plots in the field and placed in the two subplots. Genotypes were coded as to be present in subplot 1 or subplot 2. The allocation of the crops to subplot 1 or subplot 2 was randomly determined for the first plot, and the order further applied to all plots in the field. This way of allocating the crops to the subplots, results in a design where the crops are never their own neighbour (Figure 1). The number of replicates of each genotype dictated how often each genotype was present in field and how many genotypes of each crop could be “grown” simultaneously.

1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
33	45	17	4	1	6	24	40	26	24	43	13	35	11	16	41	37	16	29	45	32	12	47	20	19	1	46	3	3	17
41	46	38	35	33	31	19	18	40	2	14	23	30	36	4	12	18	44	4	42	32	20	43	40	16	4	36	30	22	31
10	19	8	15	10	22	28	17	8	4	15	45	6	16	34	7	15	25	24	15	29	9	39	33	46	14	2	16	29	18
29	42	26	2	31	5	30	11	17	41	48	36	4	47	40	30	5	29	16	37	11	45	40	44	32	36	11	44	27	13
42	23	3	23	6	10	36	23	36	33	22	4	25	6	34	26	24	34	27	36	9	21	3	27	41	39	24	20	45	46
8	28	45	30	5	32	13	13	26	1	28	29	10	25	18	39	34	8	16	43	44	30	10	21	20	14	44	34	15	47
19	22	48	13	30	28	11	10	19	34	12	32	31	8	20	38	8	40	11	24	7	9	14	25	26	8	39	19	21	32
13	34	16	22	33	48	15	4	41	20	25	15	7	37	48	48	45	19	12	6	31	42	23	9	32	9	6	28	35	31
35	41	23	17	5	37	1	11	1	29	12	34	8	5	40	10	45	22	3	7	39	22	14	18	21	20	27	21	20	32
2	38	18	14	22	11	11	12	42	15	28	24	2	2	29	38	34	21	21	3	25	39	31	38	9	15	43	48	45	5
35	7	42	37	32	31	4	2	38	28	22	43	44	35	22	43	46	45	7	12	24	43	33	16	17	14	18	8	43	24
30	13	27	12	44	1	31	26	48	1	48	38	15	30	23	46	20	46	39	39	25	18	44	31	37	17	46	33	34	26
5	33	6	10	42	6	7	39	1	7	27	44	47	47	41	27	47	14	7	1	21	37	47	10	35	47	1	7	37	48
38	36	12	33	38	8	28	43	2	27	23	47	13	40	9	23	25	32	26	44	43	26	42	46	37	5	3	19	38	24
23	27	13	21	40	9	36	35	36	3	30	18	39	29	2	19	20	25	19	28	41	40	12	25	17	5	46	29	47	6
37	35	5	42	17	2	4	41	13	27	18	41	9	16	21	17	33	35	9	3	14	48	6	26	28	11	10	3	14	42

Figure 1. Field layout. Coloured squares represent subplots. Blue squares indicate subplots containing crop 1. Grey squared indicate subplots containing crop 2. The shading and number inside the subplot indicate which genotype is allocated to the subplot. Bold black lines indicate the border of a strip. Strip number is shown atop of the field plot.

Step 2:

Data simulation

The sum of the base yield, DGE, IGE (of the neighbouring subplot(s)), strip effect, row effect, and random plot level effects made up the final yield. Yield data was simulated for each subplot. Subplots in the border strips (strip 1 and strip 15) were coded to neighbour a hypothetical crop with the IGE set to 0. For all other subplots the social effects were summed for both the left and right neighbour. No distinction was made between social effects acting within or across neighbouring strips.

Crop and field effects were drawn from various distributions, with pre-set mean and variance. The variance components of the DGE, IGE, and plot level disturbance were generated by multiplying the base yield of the recipient crop (muD) with a known scaling factor (Table 1).

Scaling factor	**Abbreviation**
DGE scaling	s_DGE
IGE scaling	s_IGE
Plot level error scaling	s_oe

The base yield for both crops were generated by drawing two numbers from the uniform random distribution, U[50, 75]. Values for the DGE were drawn from a normal distribution, N(0, s_DGE*muD). For each genotype, the genotypic IGE was calculated based on a multivariate normal distribution. Hereto first a covariance matrix (v) was created, representing the correlation of IGE of a donor crop on the recipient crop. Next, two numbers were drawn from a bivariate normal distribution, MVN (0, v). Each drawn number corresponding to a recipient crop. The generated numbers were multiplied by s_IGE and the base yield of the recipient crop, yielding the final genotypic IGE. The scaling factor for both DGE and IGE were allowed to range between 0.01 and 0.2, by increments of 0.05. 0.2 was set as maximum value, as raising either s_DEG or s_IGE above 0.2 resulted in negative yields and was therefore considered to be unrealistic for true world conditions.

Environmental effects consisted of strip, row, and random field effects. 15 strip and 16 row effects were drawn from a normal distribution, N(0, 1.5). Additional field disturbances were assigned to each subplot at the last step of the simulation. Random field effects were drawn from a normal distribution, N(0, s_oe*muD).

The final yield of each subplot was added to the field design data containing the field layout and crop and genotypic identity.

The muD, DGE, and IGE were stored in a list, serving as truth in the evaluation of the accuracy of the estimates returned by the mixed effects model.

Step 3

Extraction of the estimates

The simulated trial data was fed into a mixed effects model to extract the variance components and DGE and IGE estimates making up the final yield of a genotype. A separate analysis was run for each crop. Hereto, additional information on neighbouring crop and genotype of each subplot was added to the trial data. Next, the data was separated into two data frames, one for each crop. A dummy data frame containing the neighbour effects in a set order was added as to ensure correct coding of the factor levels in the mixed model analysis. The final crop data was analysed through mixed effects modelling using ASReml-R (Butler, 2023). The genotypic DGE ($\underline{G}_i$), and IGE ($\underline{S}_k$ & $\underline{S}_l$) were coded as

random factors in the model. The overall mean (μ) represented the base yield of the crop. The genotype of the left and right neighbour was coded as combined factor in the analysis. This way of coding enables the extraction of their individual effects. Spatial row ($\underline{r_m}$), strip ($\underline{c_n}$), and random field disturbances ($\underline{e_{ijklmn}}$) were accounted for as random effects.

$$Y_{ijklmn} = \mu + \underline{G_i} + \underline{S_{k\_left}} + \underline{S_{l\_right}} + \underline{r_m} + \underline{c_n} + \underline{e_{ijklmn}},$$

$\underline{G_i} \sim N(0, \sigma^2)$,

$\underline{S_{k\ left}} \sim N(0, \sigma^2)$,

$\underline{S_{l\ right}} \sim N(0, \sigma^2)$,

$\underline{r_m} \sim N(0, \sigma^2)$,

$\underline{c_n} \sim N(0, \sigma^2)$,

$e_{ijklmn} \sim N(0, \sigma^2)$

Step 4

Evaluation of the results

The variance components were extracted from the model, after which the estimated IGE and DGE, represented by the best linear unbiased predictors (BLUP's), and the best linear unbiased estimates (BLUE's) of fixed effects were generated and linked to their true values. The variance components were used to calculate the broad sense heritability, which was visualized using scatterplots. Scatterplots of BLUPs and their true values were next generated, and a linear model was fitted to the data to determine the regression and correlation of coefficient (adjusted R-square). The DGE and IGE estimates were then used to calculate the realized genetic gain, which was presented using boxplots.

Scenarios

Broad sense trial heritability

A simplified function was used to simulate the direct and social effects. In this function, the muD of both crops was set to the mean of the range of potential muDs. This simplified generation of base yield values, allowed for a more straightforward calculation of true variance components. The true variance components of the DGE and IGE were calculated and compared to their mean estimated values provided by ASReml-R. The broad sense heritability was calculated by dividing the variance of the term of interest (DGE or IGE), by the sum of the random error variance and variance of the term of interest. The estimated broad sense heritability for the DGE and IGE were visualized using boxplots and scatterplots. The true H^2 of the DGE and IGE were included as horizontal lines to the boxplot.

To determine how variability in DGE or IGE affect the H^2, the variance of both DGE and IGE were individually scaled using the same scaling factor as applied for the determination of the accuracy of the estimates. Other crop, field, and design parameters were kept constant. For each scaling step, the true and estimated H^2 were generated.

Next, the DGE and IGE were scaled at the same value using the set of prior stated scaling factors. For each scaling step, the H^2 was calculated over trials with increased environmental disturbance or for trials with increased number of genotypic replicates. Hereto, the same approach was followed as used during the evaluation of estimation accuracy over increased field disturbance and different number of genotypes. Increased environmental disturbance was generated by scaling the error variance to the base yield of each subplot, using the following scaling (s_oe: 0.01, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4). The trial was run with the following number of genotypes: 12, 15, 16, 18, 20, 24, 36, 48, 60, 80, 120. Scatterplots were generated for each DGE and IGE scaling, visualizing the H^2 of the DGE and IGE against the error scaling or number of genotypes.

Accuracy of the estimates

The bias (regression slope) and precision (adjusted R-square) of the model estimates was first determined under various levels of DGE or IGE. Other crop, field, and design parameters were kept constant.

Next, both IGE and DGE variance were scaled at the same value. At each step of scaling, the effect of increasing the environmental disturbance was investigated. The adjusted R-square was calculated for each scaling of the IGE, DGE, and base yield for all error terms. The adjusted R-squares of the different crop parameters were then plotted against their corresponding error scaling values.

Finally, the adjusted R-square of the DGE, IGE, and base yield was calculated and plotted for the DGE and IGE scaled at the same value, for the same number of trialled genotypes as mentioned above. The same plotting method was used as previously described.

Realized genetic gain.

The realized genetic gain was computed for each combined IGE and DGE variance scaling. The realized genetic gain was calculated by summing the estimated IGE and DGE, selecting the 10% best scoring genotypes, and extracting the sum of their true DGE and IGE. The realized genetic gain, represented by the sum of the true DGE and IGE, was calculated for scenarios with either increased field disturbance or increased number of genotypes. The realized genetic gain was calculated 10 times for each level of field disturbance or number of genotypic replicates, for each level of simulated genetic effects. The data of the ten samples containing the genetic gain were plotted against the number of replicates or error scaling using boxplots. Tukeys Least Significant Differences (LSD) were calculated to detect structural differences in genetic gain between trials with different levels of environmental disturbance or different number of genotypes.

Results

BROAD SENSE HERITABILITY

Model performance with increased DGE

Both the H^2 (E) and the H^2 (T) increase when the DGE is raised (Table 1). When comparing the H^2 (E) with the H^2 (T), small DGEs (s_DGE < 0.1) may be slightly underestimated. Increasing the magnitude of the DGEs does not seem to influence the accuracy and size of the IGE H^2 (s_DGE= 0.01: H^2 (E) (IGE) =0.20 & H^2 (T) (IGE) =0.20; s_DGE=0.05: H^2 (E) (IGE) =0.20, H^2 (T) (IGE) =0.20).

The standard deviation (SD) of the DGE H^2 appears to increase minorly when s_DGE is raised from 0.01 to 0.05 (Table 1). From intermediate DGEs (s_DGE=0.1), the SD seems to remain between 0.07 – 0.08. The SD of the IGE H^2 (E) remains between 0.05 – 0.06, cross all levels of s_DGE.

Table 1. True and estimated mean broad sense heritability under increasing levels of DGE. Standard deviations (SD) of the estimated heritability are calculated based on 100 simulations. Other model parameters are set to their default values (Appendix A, Table 1A)

s_IGE	**0.01**			**0.05**			**0.1**			**0.15**			**0.2**		
	H^2(E)	H^2(T)	SD	H^2(E)	H^2(T)	SD	H^2(E)	H^2(T)	SD	H^2(E)	H^2(T)	SD	H^2(E)	H^2(T)	SD
DGE crop 1	0.04	0.00	0.04	0.06	0.14	0.06	0.19	0.20	0.08	0.36	0.36	0.08	0.51	0.50	0.04
DGE crop 2	0.03	0.00	0.03	0.06	0.014	0.06	0.20	0.20	0.07	0.37	0.36	0.08	0.50	0.50	0.03
IGE crop 1	0.20	0.20	0.05	0.21	0.20	0.05	0.21	0.20	0.06	0.19	0.20	0.06	0.20	0.20	0.20
IGE crop 2	0.20	0.20	0.06	0.20	0.20	0.06	0.20	0.20	0.06	0.19	0.20	0.06	0.20	0.20	0.20

Model performance with increased IGE

The same pattern as found under increased levels of DGE appears present when the IGE becomes larger (Table 2). The same pattern holds true for both bias and SD.

Table 2. True and estimated mean broad sense heritability under increasing levels of IGE. Standard deviations (SD) of the estimated heritability are calculated based on 100 simulations. Other model parameters were set to their default (Appendix A, Table 1A)

s_IGE	**0.01**			**0.05**			**0.1**			**0.15**			**0.2**		
	H^2(E)	H^2(T)	SD	H^2(E)	H^2(T)	SD	H^2(E)	H^2(T)	SD	H^2(E)	H^2(T)	SD	H^2(E)	H^2(T)	SD
DGE crop 1	*0.14*	*0.14*	*0.07*	*0.14*	*0.14*	*0.08*	*0.14*	*0.14*	*0.07*	*0.14*	*0.14*	*0.09*	*0.13*	*0.14*	*0.08*
DGE crop 2	*0.13*	*0.14*	*0.06*	*0.14*	*0.14*	*0.06*	*0.14*	*0.14*	*0.08*	*0.14*	*0.14*	*0.07*	*0.14*	*0.14*	*0.08*
IGE crop 1	*0.01*	*0.05*	*0.02*	*0.07*	*0.14*	*0.04*	*0.19*	*0.20*	*0.06*	*0.36*	*0.36*	*0.07*	*0.48*	*0.50*	*0.07*
IGE crop 2	*0.01*	*0.05*	*0.02*	*0.06*	*0.14*	*0.04*	*0.19*	*0.20*	*0.05*	*0.35*	*0.36*	*0.06*	*0.50*	*0.50*	*0.07*

Plot level disturbance

Increasing the random field disturbance seems to decrease the trial heritability exponentially irrespective of the magnitude genetic effects (Figure 2A-E). Larger DGEs and IGEs appear to decrease the rate of H^2 decline (Figure 2A-E). The DGE H^2 and IGE H^2 decline at similar rates, regardless of the amount of DGE, IGE, or environmental error (Figure 2A-E). The heritability appears to be estimated with little to no bias cross all levels of environmental and genetic effects (Figure 2A-E).

Number of genotypes

Increasing the IGE and DGE results in larger H^2, irrespective of the number of genotypes (Figure 2A-E). Decreasing the number of genotypes does not seem to influence the H^2 (Figure 2A-E). The heritability estimates appear unbiased for all number of trialled genotypes when genetic effect sizes are below their maximum (s_DGE & s_IGE = 0.2) (Figure 2A-D). When 12 genotypes are trialled under largest effect sizes, the heritability may be slightly underestimated (Figure 2E).

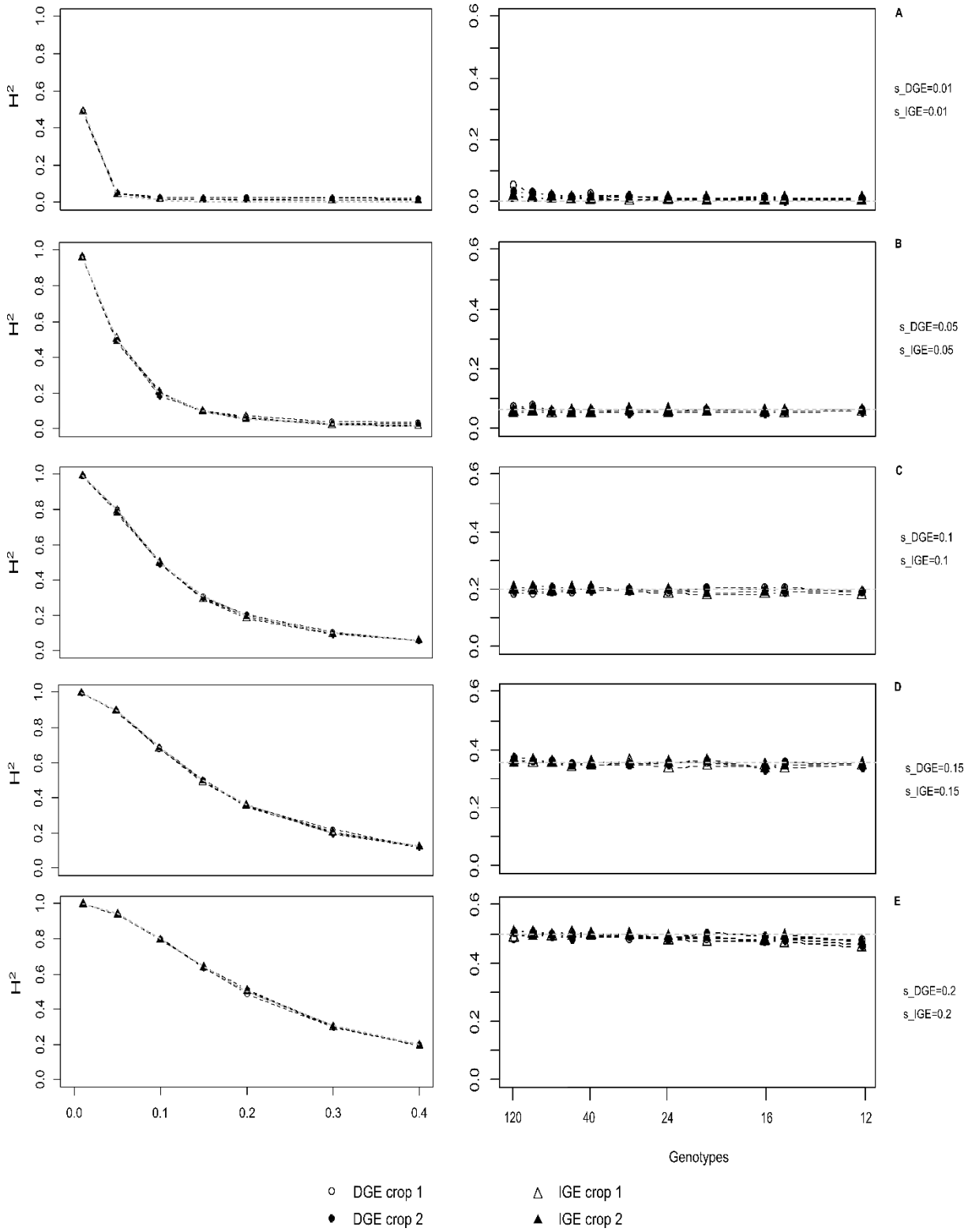

Figure 2. Estimated broad sense heritability for varying amounts of DGE and IGE, under increased field disturbances or with varying number of genotypes. Open symbols represent crop parameters of crop 1. Coloured symbols represent parameters of crop 2. Circles indicate the estimated DGE. Triangles indicate estimated IGE. Simulated heritability is represented by dashed line with (x) under increased field disturbance. Grey dashed line represents the simulated heritability.

ACCURACY OF THE ESTIMATES

Model performance with increasing DGE

When increasing the DGE, the coefficient of determination (r^2) between true and estimated DGE increases from 0.0 to 0.76, respectively (Table 3). Raising s_DGE from 0.01 to 0.2 seems to coincide with a decreased r^2 for the IGE (0.61 to 0.56 and 0.60 to 0.54 for crop 1 and crop 2, respectively). The base yield r^2 of crop 1 and crop 2 does not appear to be affected by larger DGEs and remains around 0.9.

At intermediate DGEs (s_DGE = 0.1), the regression slope nears the identity line. Further raising the DGE to its maximum value (s_DGE = 0.2) does not appear to improve the fit of the slope (Table 3).

Table 3. Regression and adjusted R-square of the different model components under varying levels of DGEs. Other model parameters were kept at their default value (Appendix A, Table 1A).

s_DGE	**0.01**		**0.05**		**0.1**		**0.15**		**0.2**	
	slope	r^2.adj	slope	r^2.adj	slope	r^2.adj	slope	r^2.adj	slope	r^2.adj
Base yield crop 1	*0.94*	*0.94*	*0.90*	*0.94*	*0.95*	*0.93*	*0.90*	*0.90*	*0.90*	*0.94*
Base yield crop 2	*0.94*	*0.94*	*0.98*	*0.93*	*0.95*	*0.92*	*0.89*	*0.91*	*0.95*	*0.91*
DGE crop 1	*0.04*	*0,00*	*0.55*	*0.12*	*0.94*	*0.39*	*1.00*	*0.64*	*0.99*	*0.76*
DGE crop 2	*0.04*	*0.00*	*0.46*	*0.12*	*0.98*	*0.39*	*0.97*	*0.62*	*1.00*	*0.76*
IGE crop 1	*0.95*	*0.61*	*1.00*	*0.59*	*0.98*	*0.56*	*0.99*	*0.56*	*1.01*	*0.56*
IGE crop 2	*0.98*	*0.60*	*0.98*	*0.59*	*0.98*	*0.58*	*0.95*	*0.56*	*0.98*	*0.54*

Model performance with increasing IGE

Raising s_IGE from 0.01 to 0.2 comes with an increased r^2 (IGE crop 1: 0.00 to 0.84; IGE crop 2: 0.01 to 0.84) (Table 4). An opposite trend appears to be present for the DGE and base yield r^2 when s_IGE is increased (DGE crop 1: 0.47 to 0.37; DGE crop 2: 0.47 to 0.38). Raising the IGEs appears to reduce the correlation coefficient of the base yield (r^2 crop 1: 0.98 to 0.85; r^2 crop 2: 0.95 to 0.83) (Table 4).

The slope of the IGE regression line for both crop 1 and crop 2 steeply increases when the IGE is enlarged from the smallest effect size (s_IGE = 0.01) to slightly larger effects (s_IGE=0.05) (r^2 IGE crop 1: 0.11 - 0.83; r^2 IGE crop 2: 0.08 - 0.82). The regression line of the IGE follows the identity line from s_IGE=0.1 onwards for both crop 1 and crop 2. A contrasting trend appears to be present for regression slope of the base yield of crop 1 and crop 2. Raising the IGE from its smallest to its largest values, reduces the slope fit by 0.19 for both crop 1 and crop 2.

Table 4. Regression slope and adjusted R-square of the different model components under varying levels of IGE. . Other model parameters were kept at their default value (Appendix A, Table 1A).

s_IGE	**0.01**		**0.05**		**0.1**		**0.15**		**0.2**	
	slope	r^2.adj	slope	r^2.adj	slope	r^2.adj	slope	r^2.adj	slope	r^2.adj
Base yield crop 1	*0.99*	*0.98*	*0.94*	*0.96*	*0.94*	*0.92*	*0.88*	*0.88*	*0.80*	*0.85*
Base yield crop 2	*0.97*	*0.98*	*0.96*	*0.97*	*0.91*	*0.93*	*0.88*	*0.88*	*0.78*	*0.83*
DGE crop 1	*0.95*	*0.47*	*0.96*	*0.42*	*0.94*	*0.40*	*0.92*	*0.38*	*0.88*	*0.37*
DGE crop 2	*0.93*	*0.47*	*0.94*	*0.45*	*0.96*	*0.42*	*0.94*	*0.38*	*0.95*	*0.38*
IGE crop 1	*0.11*	*0.01*	*0.83*	*0.23*	*1.01*	*0.58*	*1.00*	*0.74*	*1.00*	*0.84*
IGE crop 2	*0.08*	*0.00*	*0.82*	*0.26*	*1.01*	*0.58*	*1.00*	*0.75*	*1.00*	*0.84*

Plot level disturbance

DGE and IGE

Estimating the DGE and IGE of crops with minor social and direct genetic effects (s_DGE = 0.01; s_IGE = 0.01), gives accurate results for IGE and DGE (r^2 ~ 0.75) when the field disturbance is low (s_oe = 0.01). Increasing the error term asymptotically decreases the correlation coefficient of both DGE and IGE (Figure 3A). Raising the DGE and IGE appears to reduce the speed at which the correlation coefficient of both IGE and DGE decline when the field disturbance increases in magnitude (Figure 3A-E). Taking greater values for the IGE and DGE seems to result in different rates of r^2 decline between DGE and IGE (Figure 3B-D). The r^2 of the DGE appears to decline at a higher rate, compared to the r^2 of the IGE. The coefficient of correlation of both IGE and DGE however seem to reach the same asymptote at heavy field disturbance (s_oe = 0.4) (Figure 3A, C). The decline in r^2 of both IGE and DGE appears to be asymptotically at small to intermediate simulated effects (s_DGE/ s_IGE =0.01; s_DGE/ s_IGE = 0.1) (Figure 3A-C). With larger genetic effects, the decline in correlation between true and estimated values seems to follow a more linear trend (Figure 3D, E).

Base yield

The correlation between true and estimated base yield effects for crop 1 and crop 2 appears to remain constant irrespective of the magnitude of the field disturbance when minor to moderate IGE and DGE are modelled (s_DGE/ s_IGE = 0.01 & s_DGE/ s_IGE = 0.1) (Figure 3A-C). Running the same model with larger DGE and IGE seems to reduce the r^2 of the base yield for both crops (Figure 3D, E). The correlation coefficient however appears to be unaffected by the magnitude of field disturbance.

Number of genotypes

IGE and DGE

Increasing the number of genotypic replicates does not appear to influence the accuracy of the estimates at the lowest IGE and DGE scaling (s_DGE & s_IGE = 0.01) (Figure 3A). The DGE and IGE are more poorly estimated, with values close to 0, irrespective of the number of genotypes. At DGE and IGE values above moderate (s_DGE & s_IGE > 0.1), the r^2 of the base yield is estimated with less accuracy compared to the r^2 of both DGE and IGE, for trials simulated with less than 30 genotypes (Figure 3D, E).

Base yield

The r^2 for the base yield seems to be accurately estimated for all trialled number of genotypes (r^2 ~ 1) when the IGE and DGE are at their smallest value (s_DG & s_IGE = 0.01). Increasing the DGE and IGE, while reducing the number of genotypes appears to result lower estimation accuracy of the base yield (Figure 3B-E). Under moderate to large DGE and IGE (s_DGE & s_IGE = 0.1 - 0.2), the estimation accuracy seems to fluctuate between both crops for the same number of genotypes tested (Figure 3C-E).

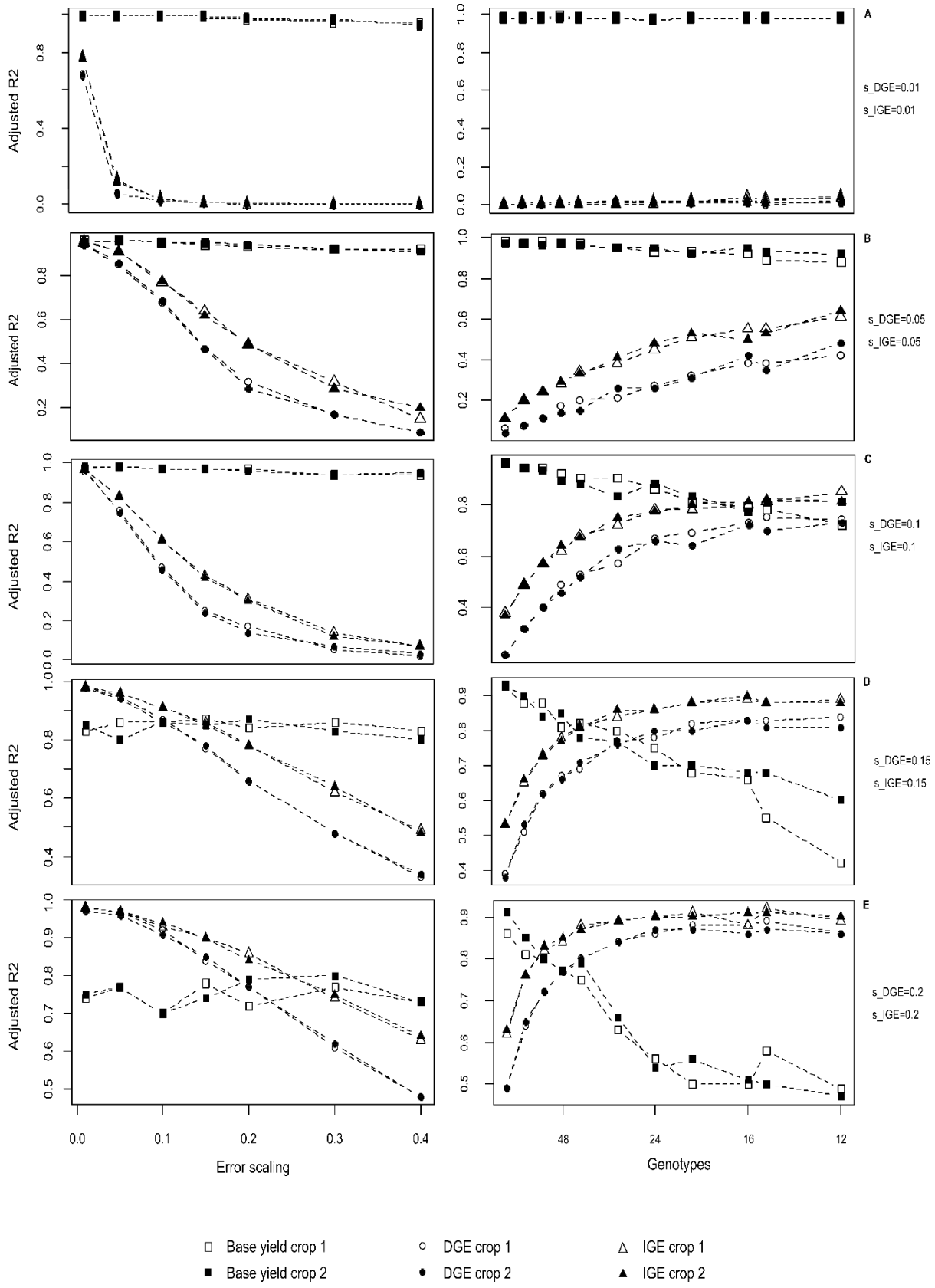

Figure 3. Correlation between true and estimated effects for varying amounts of DGE and IGE, under increased field disturbances or with varying number of genotypes. Open symbols represent crop parameters of crop 1. Coloured symbols represent parameters of crop 2. Squares indicate the estimated base yield values. Circles represent the estimated DGE. Triangles indicate estimated IGE.

REALIZED GENETIC GAIN

Plot level disturbance

Larger field disturbances appear to reduce the genetic gain irrespective of the magnitude of genetic effects (Figure 4A-E). Larger genetic effect sizes seem to result in greater genetic gain (Figure 4A-E). The decline in genetic gain under greater field error is relatively lower when the genetic effects are increased (Figure 4A-E). When the DGE and IGE are set to their minimal effect size (s_DGE & s_IGE= 0.01), only scenarios with minimal environmental disturbance (s_oe=0.01) consistently significantly differ (significant differences for both crop 1 and crop 2) from the other scenarios with simulated error (Figure 4A). Slightly increasing the genetic effects (s_IGE & s_DGE= 0.05) gives more contrasting results for the different levels of field disturbance (Figure 4B). Crop 1 and crop 2 are assigned to different groups. Significance levels however still overlap between groups of both crops. The number of treatment groups becomes smaller with large DGE and IGE (s_DGE & s_IGE = 0.05: 9; s_DGE & s_IGE = 0.2: 5) (Figure 4B, E). Increasing the genetic effect size appears to reduce the skewness of the data (Figure 4A-E).

Number of genotypes

The genetic gain appears to be more precisely estimated when the number of genotypes becomes larger (Figure 4A-E). The difference in precision between trials with contrasting number of genotypes seems to be larger when the genetic effect sizes are small (s_DGE & s_IGE< 0.1). Increasing the number of genotypes does not seem to give consistently significantly different results in terms of genetic gain (Figure 4A-E). Larger genetic effect sizes however appear to result in greater genetic gain, when comparing between similar sized trials with the same field disturbances (Figure 4A-E).

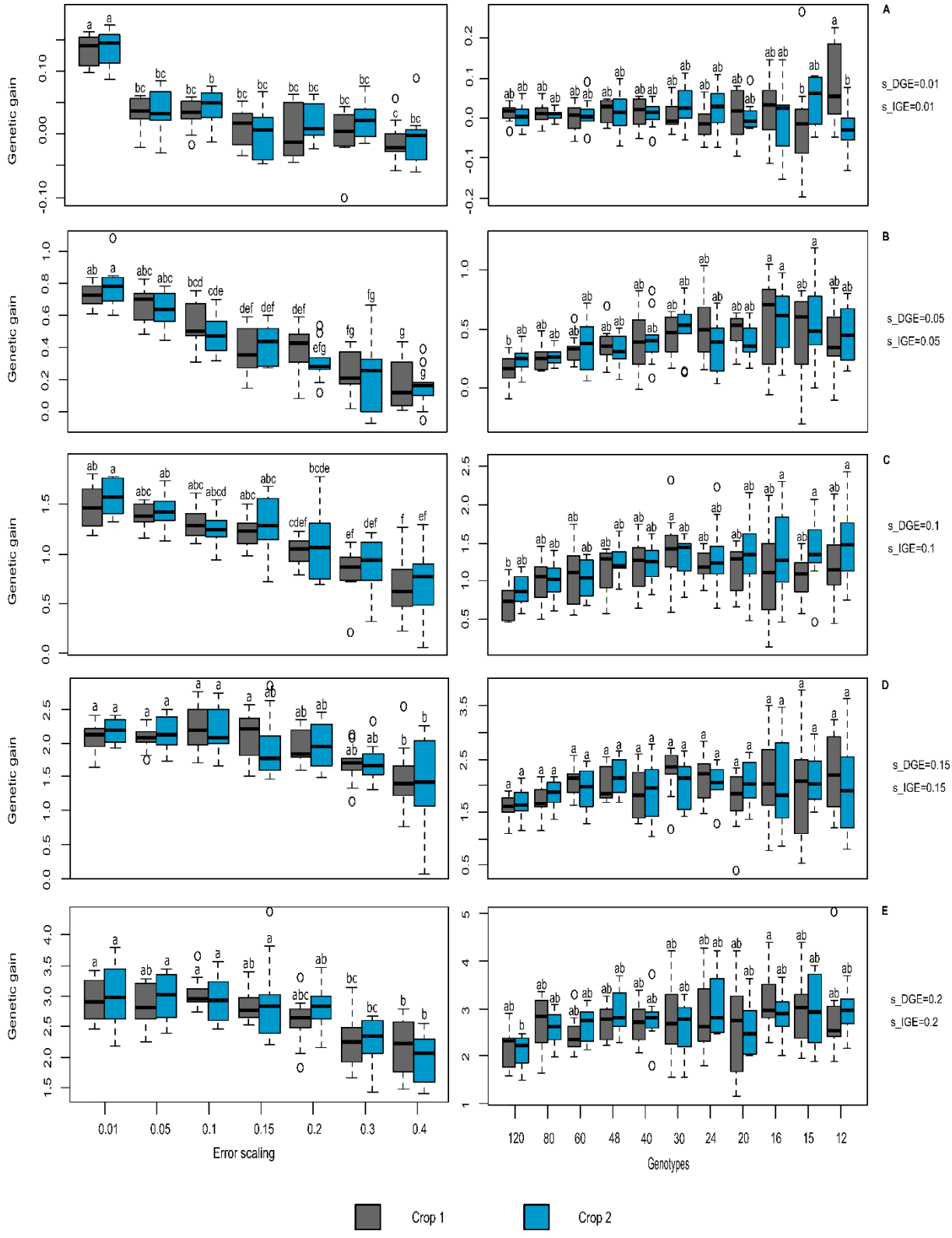

Figure 2. Predicted genetic gain for varying amounts of DGE and IGE, under increased field disturbances or with varying number of genotypes. Grey boxes represent crop 1. Blue boxes represent crop 2. Letters indicate groups of significant differences based on Tukeys LSD.

Discussion

BROAD SENSE HERITABILITY

Increasing the genetic effects was hypothesised to result in less biased heritability estimates. Our results indicate little bias irrespective of the magnitude of genetic effects. The model appears to be estimating the variance components with enough precision to accurately calculate the heritability of the genetic components.

We expected larger field disturbances to give more biased heritability estimates. We found little bias of the estimated H^2 irrespective of the magnitude of plot level disturbance. The model appears suitable for the estimation of the H^2 over varying levels of environmental disturbances.

We hypothesised to see increased heritability bias when the number of trialled genotypes was reduced. We found no interaction between number of trialled genotypes and bias of the heritability, except when largest genetic effects were simulated for trials with the smallest number of trialled genotypes. In this scenario, a minor downward bias was observed. Six levels is indicated to be the minimum number of levels required for a random term in mixed effects modelling (Cornwell & Krantz, 2014). In our study, twelve was set as minimum number of genotypes to be included in the trial. Reducing the number of trialled genotypes below twelve, may further increase the bias of the heritability estimates.

ACCURACY OF THE ESTIMATES

It was hypothesised that increasing the DGE and IGE would reduce the bias and SE of the estimates. We observed a more complex response in terms of bias and precision of the true and estimated effects when the magnitude of the DGE or IGE was raised. Raising either the DGE or IGE seemed to improve the bias and precision of the parameter of interest but reduced the precision and bias of the other model parameters, including the intercept. The trade-off between improved accuracy of the DGE and IGE, and decreased accuracy of the intercept may be explained by the ratio of the between group sample size (number of genotypes, n=48) and magnitude of simulated DGE and IGE. Random effects are assumed to have a mean of zero in ASReml-R (Gilmour, Gogel, & Welham, 2015). Increasing the scaling factor of the DGE or IGE increases the variation of the genetic effects, which by chance may have shifted the sample mean away from the population mean of zero. This could have result in inaccurate estimation of the intercept (base yield). Mixed effects models are however indicated to be robust and provide reliable estimates even when model assumptions are violated (Schielzeth et al., 2020). (Knief & Forstmeier, 2021) however indicated that parameter estimates were indeed unbiased at large sample sizes but became more biased when sample sizes decreased.

Variability in IGE appeared to have a stronger negative effect on the other model terms, than variability in DGE. The negative effect of increased IGEs affected both the bias and precision of the other model parameters, while variability in DGE mainly appeared to reduce the precision. A genotype has one record expressing the DGE, while the IGE of a genotype is expressed twice. The IGE may therefore be expected to give more “noise” to the data, giving a stronger response on the other model parameters.

It was further expected that larger environmental disturbances decrease the precision of the estimates, which is partly supported by our findings. The precision of the random model terms appears to reduce when the environmental error is larger. The rate of decline is however dependent on the magnitude of the genetic effects.

The base yield appeared to be unaffected by field disturbances. The model intercept is only dependent on the sample mean of the variance components. According to the central limit theorem, the sample mean more closely follows the true population mean when the sample size increases (Fischer, 2011). With 480 subplots, the sample mean of the environmental disturbance may remain close to the population mean of zero, even when the effect size increases.

We hypothesised to see more precise estimates of the genetic effects when the number of genotypic replicates was increased. Our findings suggest that the precision of the DGE and IGE estimates follow this behaviour. The base yield accuracy however appears to show an opposite trend and show reduced precision with more genotypic replicates (fewer number of trialled genotypes). The reduced trend appeared most apparent when genetic effects were large. This negative effect on the base yield could again be explained by the sample mean of the variance components moving away from the true population mean of zero when the number of genotypic replicates is decreased.

REALIZED GENETIC GAIN

We expected the realized genetic gain to increase with larger simulated genetic effects and decrease with greater environmental disturbance. Our results underline these hypotheses. Our results indicate that there is an interaction between the level of genetic effects and the magnitude of environmental disturbances. Enlarging the genetic effects appeared to give more distinct results in terms of genetic gain under increasing field disturbance. Most distinct differences between trials with different levels of field error were however found for trials simulated with intermediate levels of genetic effects. Trials run with large genetic effects showed constant genetic gain, until field errors neared their maximum values. These observations may be explained by the accuracy of the underlying estimates. Under intermediate effects, the accuracy of the estimates decreases with larger field disturbances. The estimated top performing genotypes may not always be the true best performing genotypes, resulting in lower genetic gain. Under maximum genetic effects, the IGE and DGE can be estimated with enough accuracy to result in the estimated best performing genotypes to match the true best performing genotypes. Estimated and true top performing genotypes may only start to differ when field disturbances near their maximum value.

We further predicted to obtain higher genetic gains when the number of trialled genotypes was increased. No significant differences in magnitude of genetic gain were found when the number of genotypes in the field was increased. Our data however suggest a larger spread in realized genetic gain when the number of trialled genotypes is brought down. Running a trial with few genotypes may lead to the field variability in DGE and IGE not fully representing the potential total variability. Furthermore, the amount of sample variation for the DGE and IGE may not be the same in each simulation. Fluctuations in the sample variation for DGE and IGE may be reflected in the spread of the realized genetic gain for trials with reduced number of genotypes.

Finally, our findings suggest that the distribution of realized genetic gains may be skewed when genetic effects are minor and/or environmental disturbances are increased. The skewness may be caused by a combination of factors. Factors including the inaccurate estimation of the genetic effects, small sample size underlying the predicted genetic gain, and the limited number of data points used to construct the boxplots. Due to computational constraints, no more than 10 data points per box were generated in this study. To gain stable means, a minimum of 85 samples is however recommended (Piovesana & Senior, 2018). It may therefore be recommended to construct the boxplots with at least 85 data points per box.

Recommendations

In this study no distinction between social effects coming from left or right neighbours were made. In real world settings, systemic differences between being a right or left neighbour may be present. Directional factors such as shading, where being a right or left neighbour might have consistent negative effects when the sun is coming from the right- or left-hand side. Future studies may introduce directionality to the data by means of a directional scaling factor and investigate how well the model may be able to account for the directional noise. Since in a real-world trial, it is not known to what extent directional effects are present, it may be worth to first investigate how well the model may be able to estimate the magnitude of the directional scaling factor. The trial data may then be analysed using the estimated scaling factor as cofactor, and the model performance evaluated.

We further did not investigate how well the model may be able to account for strip, row, or local field effects. Trials with varying levels and types of field effects could be simulated and analysed using packages such as SpATS (Rodríguez-Álvarez, Boer, van Eeuwijk, & Eilers, 2018). SpATS allow the user to model the spatial trends and fit a mixed effects model to the trial data.

In this research, it was not studied how variation in the number of genotypes affects the heritability, genotypic estimates and realized genetic gain under different levels of field disturbance. Having a deeper understanding of the interaction between number of genotypes and field disturbances may serve useful as information on field effects may be available from earlier trials and could then be used to aid in the decision making of future trial designs.

Early in the study, we spent some time on developing a strategy to balance the occurrence of genotypic combinations in the design. Due to time constraints this field of interest was not pursued further. It may be worth to investigate whether complete random allocation of neighbours (as was the approach in this study) yields different results, compared to a more balanced approach. In a more balanced approach, the specific combinations of left neighbour, middle genotype and right neighbour should be balanced. One could use a brute force approach where large number of field designs are generated randomly and the occurrences of the specific combinations of neighbouring combinations are calculated. The designs with the most balanced occurrence of genotypic combinations may then be extracted. The standard deviation over the occurrences of the neighbouring combinations can be used as a proxy to select the most balanced designs.

We applied a field design, where the same crop was always present in the left or right subplot, yielding a design where a crop is never its own neighbour (“strip cropping”). Alternatively, crops may completely be randomized within a strip (“pixel cropping”), or the crop identity within a strip could be switched in the middle (“semi-strip cropping”). Investigating whether the different designs yield similar outcomes may also be a focus of follow up studies. The “pixel cropping” further allows for estimation of crop-to-crop effects, as crops may be their own neighbour.

The realized genetic gain was calculated for one generation. It may be interesting to study what the long-term genetic gain is for different intercropping scenarios. AlphaSim has been applied to evaluate intercropping breeding trials based on phenotypic selection and is indicated to be flexible in the trial design (Bančič et al., 2021; Gaynor, Gorjanc, & Hickey, 2020).

Conclusion

BROAD SENSE HERITABILITY

The broad sense heritability can be estimated with little to no bias irrespective of the magnitude of genetic or environmental effects, for trials run with varying numbers of genotypes. Larger genetic effects result in greater H^2, with larger environmental effects reducing the H^2.

ACCURACY OF THE ESTIMATES

Increasing the magnitude of the genetic effects, improves the precision and reduces the bias of their estimates. Larger genetic effects can however negatively affect the precision and bias of the other model parameters. The extent to which increased levels of genetic effects negatively impact the estimation of the base yield is also dependent on the number of trialled genotypes. Decreasing the number of trialled genotypes in trials with large genetic effects, worsens the precision of the base yield estimate. The base yield estimates are however not affected by increased field disturbance. Increasing the field disturbance does reduce the precision of the DGE and IGE estimates.

REALIZED GENETIC GAIN

Larger genetic effects increase the amount of realized genetic gain. The genetic gain can however significantly decrease when the environmental error is raised. Raising the number of trialled genotypes does not increase the genetic gain but results in a less varied response in terms of genetic improvement.

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Appendix A: Model parameters

Table 1A. Model variables, their abbreviations and default values.

Variable	Variable code	**Value**
Minimum direct genetic crop effect on crop 1	yield_min1	50
Maximum direct genetic crop effect on crop 1	yield_max1	75
Minimum direct genetic crop effect on crop 2	yield_min2	50

Maximum direct genetic crop effect on crop 2	yield_max2	75
Scaling factor for the direct genetic effects on the genotype	s_DGE	0.1
Scaling factor for the indirect genetic effects on the genotype	s_IGE	0.1
Correlation of the social effects of a donor genotype on the recipient crop.	cor_genos	0.8
Standard deviation of the strip effect	strpe	1.5
Standard deviation of the row effect	rwe	1.5
Error scaling factor	s_oe	0.2