# Contrasted reaction norms of wheat yield in pure vs mixed stands explained by tillering plasticities and shade avoidance

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# Abstract

- **Context**

Mixing cultivars is an agroecological practice of diversification, increasingly used for cereals. The yield of such mixtures is higher on average than the mean yield of their components in monoculture, i.e., a relative yield total (RYT) higher than 1 but with a large variance. The causal drivers of this variance are plant-plant interactions leading to phenotypic plasticity between pure and mixed stands. A prerequisite to quantify such a plasticity is the identification of each cultivar in the mixture, which is challenging and rarely done.

- **Research Question**

A mixture could under- (RYT<1) or over-yield (RYT>1) depending on the relative magnitude of phenotypic plasticities of its component cultivars. We hypothesized that the magnitude of yield plasticity of a given cultivar is associated with the magnitude of the difference between

its monoculture yield and the average monoculture yield of the other cultivars it is mixed with. Given the sequential decomposition of yield into multiplicative components, we then hypothesized that the plasticity of the first to be established, spike number, would also be the main contributor to yield plasticity.

- **Methods**

A new experimental design based on a precision sowing allowed the phenotyping of each cultivar in mixture, at the level of individual plants, for above-ground traits throughout growth. Eight commercial cultivars of winter wheat (*Triticum aestivum* L.) were grown in pure and mixed stands in field plots for two seasons with nitrogen fertilization, fungicide and herbicide treatments. Two quaternary mixtures were assembled with cultivars contrasted either for height or earliness. Plant-plant interactions in mixture were maximized by randomizing genotypes on a regular lattice.

- **Results**

Among the two mixtures, one strongly underyielded over both seasons (RYT ≅ 0.71) while the other overyielded the second season (RYT=1.11) and slightly underyielded the first season (RYT = 0.92). Six cultivars out of eight displayed a significant phenotypic plasticity for grain weight ($\alpha$=5%) and, in the second season, the magnitude of plasticity in grain weight was significantly ($\alpha$=5%) and positively associated with their relative yield differences in monoculture. In terms of yield components, the plasticity in spike number was the strongest contributor to yield plasticity (~56%), driven by even stronger but opposed plasticities in both tiller emission and regression. Significant phenotypic plasticity in height was also detected and, in both seasons, the plasticity in tiller emission was significantly, positively associated with the height differentials between cultivars in mixture ($\alpha$=5%).

- **Conclusions**

Active plasticity in the early recognition of potential competitors for resources is a major component of genotype strategies in mixtures, as shown here for tillering dynamics. Our results also highlighted a link between plasticity in tiller emission and height differential in mixture. Both height and tillering dynamics displayed convergence patterns typical of the shade avoidance syndrome.

- **Implications**

This study implies that phenotyping the maximum in tiller emission, in both pure and mixed stands, in addition to phenotyping height dynamics and spike number, will help understanding the neighborhood-dependence of life-history strategies. Methodologically, both the new experimental design and decomposition of plasticities open avenues to better study plant-plant interactions in agronomically-realistic conditions. This study also contributed a unique, plant-level data set allowing the calibration of process-based plant models to explore the space of all possible mixtures.

**Keywords**: plant-plant interactions, phenotypic plasticity, tillering, cultivar mixtures, bread wheat, yield components, shade avoidance syndrome

# Introduction

## Agroecological transition, intra-plot diversification and over-yielding in varietal mixtures

The main farming system for open-field annual species in Europe currently relies on industrial agriculture that provides high levels of productivity. This system is notably dependent on cultivars selected for their high-yield potential under optimal conditions obtained thanks to a massive use of chemical inputs (Therond et al., 2017). Besides the large increase in crop production (Evenson and Gollin, 2003), this system is associated with negative externalities. Indeed, pesticides were found to be harmful for the environment (Leenhardt et al., 2022) and human health (Baldi et al., 2013) and the massive use of chemical inputs led to soil degradation and contamination (Lal, 2007), soil salinity (Hatt et al., 2016) and water pollution (Moss, 2008), endangering food security in the long term. In contrast, agroecology promotes low-input practices (Altieri, 1989), with diversification at various spatiotemporal scales as a main lever (Gliessman, 2014). For annual crops at the scale of a plot, it consists in sowing cultivar or species mixtures (Barot et al., 2017; Justes et al., 2021) with the goal of taking advantage of niche complementarity and facilitation (Brooker et al., 2015). Both practices see a renewed interest from farmers and academics (e.g., the ReMIX project https://www.remix-intercrops.eu/). Yet, a quantitative and dynamic understanding of such mixed stands is still lacking (Gaudio et al., 2019).

Cultivar mixtures, even if relying on a lower functional diversity than species mixtures, have already demonstrated various advantages as indicated by the comparison index named “relative yield total” (RYT) which equals the total yield of the mixture divided by the yield of the cultivars in monoculture weighted by their sowing proportions in the mixture. For our case study of winter wheat, on average cultivar mixtures provide a higher yield than the mean of

their yield in pure stands (RYT > 1 meaning overyielding), +2% on average (Borg et al., 2018). This is especially observed under high disease pressure (+5% on average), a well-known "mixture" effect due to their limitation of airborne fungal epidemics (de Vallavieille-Pope, 2004; Mundt, 2002; Wolfe, 1985). Cultivar mixtures also display a better inter-annual stability than their components in pure stands (Reiss and Drinkwater, 2018). However, RYT displays a large variability, of ± 40% in the meta-analysis of Borg et al. (2018). Moreover, even under optimal conditions (fertilizers and pesticides), which we will focus on in this work, there still is a large variability in RYT between mixtures, of ± 20% in the same environment (data from Forst *et al.*, 2019). Under such optimal, yet competitive conditions, a lower yield of the mixture compared to the mean of the pure stands, i.e., underyielding (RYT < 1), would be interpreted as a tragedy of the commons (Anten and Vermeulen, 2016). For example, stem elongation in a mixture compared to its pure stands could be observed, with a side effect on yield, typical of the shade avoidance syndrome (Franklin, 2008). At the opposite, the overyielding of a mixture could be driven by an increase in frequency of the most productive cultivar in pure stands and by complementarity effects (Loreau and Hector, 2001).

## Differences in relative yields between mixtures and importance of plasticity

Deciphering the causes of RYT variability is therefore critical. First, wheat cultivars are known to display a large variability for yield (grain weight per unit area), some outperforming the others in a given environment. Hereafter, a cultivar will be said to dominate another *within* a given stand when its yield is higher, either if observed in parallel pure plots, or in the same mixture plot.

Moreover, a cultivar performance can change when observed *between* stands, in pure vs. mixed stand, whether positively or negatively. We speak here of "phenotypic plasticity" for the capacity of plants to adjust their strategy, i.e., their phenotypes, depending on their abiotic conditions and biotic neighborhood (Schmitt, 1993). Phenotypic plasticity is understood here as the tendency for phenotypic variation to be influenced by the environment (Hallgrimsson et al., 2019). As plant-plant interactions occur in both pure and mixed stands, as formalized in (Forst et al., 2019), we will consider neighborhood heterogeneity as the main environmental variable, lower in pure stands and higher in mixed stands. The reaction norm of interest here for a given cultivar will hence be its mean trait value in pure versus mixed stands.

Regarding the large variability in RYT, such gaps between observed and expected mixture yields correspond to phenotypic plasticity between pure and mixed stands. Notably, even in the case of strong dominance between cultivars in pure stands, an absence of plasticity leads to a RYT equal to unity. Indeed, the dominance between cultivars in a given mixture will depend on both the dominance between them in pure stands as well as their plasticity between pure and mixed stands, and this allows to interpret both cases defined by the deviation of RYT from unity. In the overyielding case (RYT > 1), at least one cultivar should exhibit a positive reaction norm from pure to mixed stand, and its yield gain should be higher than the eventual cumulated yield loss of the other cultivars. Oppositely, in the underyielding case (RYT < 1), at least one cultivar should exhibit a negative reaction norm, its yield loss exceeding the eventual cumulated yield gain of the other cultivars. The important factor here is the asymmetry between what is gained by a cultivar and what is lost by another, i.e., the absence of exact compensation between their reaction norms.

However, the extent of phenotypic plasticity in yield and its components between pure and mixed stands, and the magnitude of plasticity differences between cultivars, remain largely

unexplored, as well as the underlying traits and processes. Studying them also raises specific questions in terms of experimental design.

## Phenotypic integration

It is also imperative to go beyond the analysis of reaction norms for single traits separately given the importance of phenotypic integration that can lead to compensation between traits (Pigliucci and Preston, 2003). Crops such as cereals are especially well-studied in this regard. Beyond plant density, grain yield is decomposed into several components, spike density, grain number per spike and thousand kernel weight, that display contrasted phenotypic plasticities (Sadras and Slafer, 2012). For instance, the tillering process which conditions the final number of spikes of a given plant is highly plastic with respect to plant density (Darwinkel, 1978). The density of neighboring plants early in the crop cycle is perceived via a signal of light quality (red:far-red ratio, R:FR) (Demotes-Mainard et al., 2016; Smith, 2000) and exploited, as a predictor of future competition for light (Schmitt and Wulff, 1993), to adjust tillering (Casal, 1988; Evers et al., 2006; Xie et al., 2016).

Furthermore, a plant strategy involves resource allocation between vegetative and reproductive structures (Reekie and Bazzaz, 2011). Any difference between stands in reproductive allocation for a given cultivar can be due to the allometry between total and reproductive biomass, or to the plasticity of this allometry (Weiner, 2004). Either way, such trade-offs are likely to differ between pure and mixed stands, e.g., as shown with a decrease in harvest index in interspecific mixtures of increasing diversity (Chen et al, 2021).

## Hypotheses, rationale and design

Contrasts in phenology and architecture among commercial cultivars lead to differences in their ability to take advantage of the environmental resources and convert them into yield.

Differences in dominance between cultivars in pure stands hence are expected. Similarly, we also expected dominance differences within mixed stands. Moreover, given the known variability in RYT, we expected it to deviate from unity. We hence hypothesized more specifically that whether a mixture is under- or over-yielding would depend on the dominance rankings of cultivars in each stand as well as, crucially, the relative magnitude of their phenotypic plasticities.

Moreover, numerous studies showed that the higher the environmental change, the larger the phenotypic plasticity. Consequently, we hypothesized that the magnitude of a cultivar's plasticity in pure versus mixed stands is associated with the magnitude of the difference between its monoculture yield and the average monoculture yield of the other cultivars with which it is mixed.

As grain weight is decomposed into several components, its plasticity should be explained by the plasticity of these components. We hence investigated whether the plasticity of grain weight is built on the sum of smaller plasticities in yield components, or if it is buffered by opposed reaction norms of these yield components. Moreover, the yield components are established all along the crop cycle. Compared to other signals that can occur more or less late in the cycle, the presence of neighbors is being perceived early by the plants, notably through light signals. As a result, differences in neighbor heterogeneity between pure and mixed stands are more likely to disproportionately affect the first yield component, namely spike number, during tillering dynamics. We hence hypothesized that, under optimal conditions, the plasticities of branching dynamics are the main determinants of yield plasticity (Sadras and Rebetzke, 2013). Each additional tiller remaining at flowering can not only contribute to an increase in total plant biomass but also to the grain yield. We hence further hypothesized that the plasticities in yield components, and particularly spike number, should also impact the plasticity in harvest index.

The rationale of our investigation hence was to document the extent of phenotypic plasticity in pure versus mixed stands, assess its impact on the differential productivity between stands, and evaluate its genetic and environmental origins. Because we studied a strongly-selfing species, winter wheat, we could study plasticity at the within-genotype scale, and we used robust statistical procedures based on a non-parametric approach. Furthermore, stands are made of many individual plants, each adapting its strategy to its neighborhood. To reach this level of detail while maintaining agronomic relevance studying several genotypes and assemblies, we proposed a new experimental design and applied it in a field trial with eight genotypes assembled into two four-way mixtures. The innovation consisted in tracking the genotype of each individual plant in the mixed stands over their whole life cycle. The main phenological stages of each genotype were monitored. Moreover, for all individual plants, we not only assessed, at maturity, their yield components, but we also monitored, all along their growth, their height and tiller number, both traits known to be involved in light competition, to get a sense of the temporal dynamics along which plants interact. Moreover, to extend the environmental context in which such plant-plant interactions occur, we repeated our experimental study during two contrasted growing seasons.

# Materials and Methods

## Plant material

Among a panel of 210 European cultivars (Touzy et al., 2019), eight were chosen with contrasted heading dates and heights at maturity (Table S1): Accroc, Aubusson, Bagou, Belepi, Bergamo, Boregar, Expert and Kalahari. Two four-way mixtures were assembled, knowing that French farmers mix on average three to seven cultivars (Borg *et al.*, 2018). The first,

“Mixture 1”, was composed of Accroc, Aubusson, Bergamo and Expert, the first two having earlier heading dates than the two others. The second, “Mixture 2”, was composed of Bagou, Belepi, Boregar and Kalahari, with Belepi and Kalahari being taller at maturity.

## Field trial

A field trial was conducted at Le Moulon, Gif-sur-Yvette, France, from sowing on October 30, 2019 to harvest on July 20, 2020. It was fully replicated once, with sowing on November 5, 2020, and harvest on August 2, 2021. Each season, the whole trial was a rectangle of 8m wide and 12m long, and comprised 12 “nano-plots” (2m$^2$ for pure stands and 1.5m$^2$ for mixed stands): 8 pure stands and 4 mixed stands (two replicates per mixture). All nano-plots were sown as a regular lattice made of 12 ranks, with 30 rows for pure stands and 22 rows for mixed stands. Both row and rank spacings measured 8 cm corresponding to a sowing density of 160 seeds.m$^{-2}$. The spatial distribution of each mixture (Figure S1) was obtained by regularly randomizing each genotype (Lieng et al., 2012), and seeds were sown at the center of PVC rings of 0.2cm thickness, 5.5cm radius and 5cm depth (Figure S2) to facilitate the phenotyping and harvest of individual plants without disturbing their growth. In terms of agronomic management, seeds were treated prior to sowing, two fungicides as well as a herbicide were applied during the crop cycle, and nitrogen fertilization was applied twice (at the BBCH30 and BBCH55 stages). Corridors between nano-plots were regularly weeded by hand. Average daily temperature, rainfall and global radiation were measured by a local weather station and monitoring data was retrieved from the INRAE CLIMATIK platform (https://agroclim.inrae.fr/climatik/, in French) managed by the AgroClim laboratory of Avignon, France. The average daily temperature between October and August was 12.1°C in 2019-2020 and 10.8°C in 2020-2021, cumulative rainfall was 617 mm in 2019-2020 and 665

mm in 2020-2021, and cumulative global radiation was 4261.22 $MJ.m^{-2}$ in 2019-2020 and 3447.19 $MJ.m^{-2}$ in 2020-2021.

## Phenotyping

For each season, from January until June, multiple traits were phenotyped once a month. In pure stands, 20-30 plants were destructively sampled at each time point to record height (PH, in cm), tiller number (TN) and above-ground dry biomass (PW, in g) after a 48h-drying at 60°C. In mixed stands, height and tiller number were recorded non-destructively at each time point for each individual plant. Phenological stages BBCH30 (ear at 1cm) and BBCH55 (half of ear emerged above the flag leaf ligule; heading) were determined based on plants in pure stands. At harvest all remaining plants were sampled to record the following variables at the individual-plant scale: for plant i, shoot dry weight ($SW_i$, in g), spike dry weight ($SPW_i$, in g), spike number ($SN_i$), grain number ($GN_i$) and grain weight ($GW_i$). From these were then computed the total above-ground dry biomass ($PW_i = SW_i + SPW_i$, in g), the harvest index ($HI_i = GW_i / PW_i$), the grain number per spike ($GNpS_i = GN_i / SN_i$) and the thousand kernel weight ($TKW_i = GW_i / GN_i * 1000$, in g). To account for border effects, the data from all plants sown along a border of a nano-plot were discarded: only the data from inner plants were analyzed.

## Statistical analysis

### Yield

The yield of a given cultivar, whether in pure or mixed stand, was computed by dividing the mean grain weight per plant of this cultivar by the area of one plant in the stand computed as the inverse of the sowing density (1/160=0.00625 $m^2$). For French official variety trials, the GEVES accounts for border effects using inter-rank and inter-plot distances which typically

increases the microplot area used for yield calculation by 20%. Hence in our case, the area of one plant corrected for borders equaled 0.00750 $m^2$.

## Relative yield totals

The relative yield total of a given mixed stand (Reiss and Drinkwater, 2018) was computed as $RYT = Y_{mix} / (p_1 . Y_{pure,1} + p_2 . Y_{pure,2} + p_3 . Y_{pure,3} + p_4 . Y_{pure,4})$ where $Y_{mix}$ was the grain weight per plant averaged over all plants in the mixed stand, $p_g$ was the proportion of plants in the mixed stand being of genotype g, and $Y_{pure,g}$ was the grain weight per plant averaged over all plants in the pure stand of the given genotype.

## Dominance rankings between genotypes

Dominance rankings between genotypes of a given mixture were defined based on Tukey's range test on average plant grain weight using the R package multcomp (Hothorn et al., 2008). This test was applied per mixture and season. Pairwise differences between genotypes were declared significant if their p-values were below 0.05, followed by a letter-based representation (Piepho, 2004). Dominance rankings were established in ascending order based on this letter representation and decimal ranks were given to cultivars belonging to two groups. The same procedure was also applied on the pure-stand data for all genotypes of a given mixture.

## Tests of phenotypic plasticity

For each genotype per season, the following model was used: $E[y_{si}] = \mu_s$, where $y_{si}$ was the value of the trait of interest for plant i in stand s (pure or mixed), $\mu_s$ was the mean trait value in stand s and E is the expectation. The null hypothesis was that the mean of the trait in the pure stand was equal to its mean in the mixed stand: "$\mu_{pure} = \mu_{mixed}$". The hypothesis was tested separately for different traits. A bootstrap algorithm was applied, assuming neither equal variances between pure and mixed stands nor the normality of trait values per stand. One

thousand bootstrap samples were generated to approximate the distribution of the test statistic under the null hypothesis, and a p-value was computed based on this distribution. No correction was applied to correct for multiple testing over all traits and seasons as justified in an exploratory context (Heller, 2011; Rothman, 1990).

## Test of the relation between yield plasticity and yield difference between neighbors

The yield plasticity of a given cultivar in a given year, noted $pGW_{fy,}$ was quantified here using the opposite of the relative competition intensity (fifth index indicated as nr. 3 in table 1 of Weigelt and Jolliffe, 2003), i.e., $pGW_{fy} = 100 \times (GW_{mix,fy} - GW_{pure,fy}) / GW_{pure,fy}$, where $GW_{s,fy}$ is the mean grain weight of the focal cultivar *f* in stand *s* (pure or mixed) in year *y*. The same year, the relative yield difference between the focal genotype and its neighbors, noted $\Delta GW_{pure,fy}$, was quantified as the pure-stand yield of the focal cultivar *f* in comparison with the pure-stand yield of its three neighbor cultivars in the mixture, noted $mGW_{pure,y}$, with $\{n \in G \setminus f\}$ being the set of neighbors cultivars and G the set of all cultivars in the mixture:

$$mGW_{pure,fy} = 1/3 \,.\, \Sigma_{n \in G \setminus f} \; GW_{pure,ny}$$

$$\Delta GW_{pure,fy} = 100 \times ( \mid GW_{pure,fy} - mGW_{pure,y} \mid ) \,/\, mGW_{pure,y}$$

The first index was then regressed on the second one per season, using a linear model:

$pGW_{fy} = \beta_{0y} + \beta_{1y} \,.\, \Delta GW_{pure,fy} + \epsilon_f$ with $\epsilon_f \sim N(0, \sigma_y^2)$

or a quadratic model:

$pGW_{fy} = \beta_{0y} + \beta_{1y} \,.\, \Delta GW_{pure,fy} + \beta_{2y} \,.\, \Delta GW_{pure,fy}{}^2 + \epsilon_f$ with $\epsilon_f \sim N(0, \sigma_y^2)$.

Both models were compared per year using the likelihood ratio test as computed by the anova function in R.

## Decomposition of yield plasticity into the plasticity of yield components

The grain weight of a plant *i*, $GW_i$, can be decomposed into the product $SN_i$ x $GNpS_i$ x $SKW_i$ using the single kernel weight $SKW_i=TKW_i/1000$. For a given genotype in a given season, we started by summing each variable over all $n_s$ harvested plants (s indicating the stand, pure or mixed) to get $X_{s,tot}$ where X is one of GW, SN and GN, and then computed $GNpS_{s,tot} = GN_{s,tot} / SN_{s,tot}$ and $SKW_{s,tot} = GW_{s,tot} / GN_{s,tot}$. As an index of plasticity between pure and mixed stands for a given genotype in a given season, we decomposed the natural log of average GW per plant in mixture divided by average GW per plant in monoculture, $\log(GW_m/GW_p)$, into $\log(SN_m/SN_p) + \log(GNpS_{m,tot}/GNpS_{p,tot}) + \log(SKW_{m,tot}/SKW_{p,tot})$ where $SN_s$ also corresponds to the average over all plants in stand *s*.

## Plasticity in tiller emission and regression

Spike number (SN) was decomposed into a sum of two metrics, SN = MTN + (-NRT) where MTN was the maximal number of tillers and NRT the number of regressed tillers. Mean equality of each metric between pure and mixed stands was tested for each genotype and season separately with the same bootstrap approach as for yield components above.

## Correlation between tiller plasticity and height differential in mixture

For each year *y*, the height differential in mixture between genotype *g* and the other genotypes was computed at each date *d* (in days after sowing) between sowing and tiller cessation: $DeltaPH_{yg,mix}(d) = PH_{yg,mix}(d) - (\sum_{j \neq g} PH_{yj,mix}(d)) / 3$. This height differential during tiller emission was compared with a plasticity index in pure versus mixed stands for the maximal number of tillers (MTN) computed as $pMTN_{y,g} = (MTN_{yg,mix} - MTN_{yg,pure}) / MTN_{yg,pure} * 100$. Similarly, the height differential was also computed at each date *d* between tiller cessation and maturity. This index of height differential during tiller regression was compared with a

plasticity index in pure versus mixed stands for the number of regressed tillers (NRT) computed as $pNRT_{y,g} = (NRT_{yg,mix} - NRT_{yg,pure}) / NRT_{yg,pure} * 100$. For the analysis of the tiller-regression phase, we fitted three linear models: the first with only the height differential, the second adding the plasticity index for tiller emission, and the third with also the interaction between both explanatory factors. The best model was selected according to a likelihood ratio test.

### Reproducibility

Analyses were conducted in R (R Core Team 2020) and figures were produced with the ggplot2 package (Wickham, 2009). Supplementary information, data and code that support the findings of this study are openly available at the INRAE space of the *Recherche Data Gouv* repository https://doi.org/10.57745/LZS8SU.

# Results

## Significant yield plasticity in pure versus mixed stands led to dominance relationships in mixture

The average yield in our field trials equaled 93 $qt.ha^{-1}$ (111.5 $qt.ha^{-1}$ before correction for border effects), associated with an average ear density of 593 $spikes.m^{-2}$. The first season (103.1 $qt.ha^{-1}$; 630 $spikes.m^{-2}$) was more productive than the second season (83.2 $qt.ha^{-1}$; 555 $spikes.m^{-2}$). Looking at pure stands, this yield decrease was especially strong for all four cultivars of Mixture 1 (-17%) and two cultivars of Mixture 2 (-32%). Regarding mixed stands, the total yield of Mixture 2 sharply decreased the second season compared to the first (-41%) whereas the yield of Mixture 1 remained stable (-1%). The lower yields in the second season were associated with lower global radiations starting after BBCH30 as well as lower rainfall between BBCH30 and BBCH77 and higher rainfall after BBCH77 (Figure S3).

In the four mixture-season combinations, the yield observed in the mixture deviated from the expectation based on the monoculture yields and the sowing proportions, as shown by the reaction norms between pure and mixed stands (Figure 1). Three RYTs were negative, ranging from 0.60 to 0.92, and one was positive at 1.11. Mixture 1 performed globally well, with a positive RYT the second season (ear density of 577 spikes.m$^{-2}$ both seasons), whereas Mixture 2 was underyielding both seasons (with 687 spikes.m$^{-2}$ in 2019-2020 and 529 spikes.m$^{-2}$ in 2020-2021).

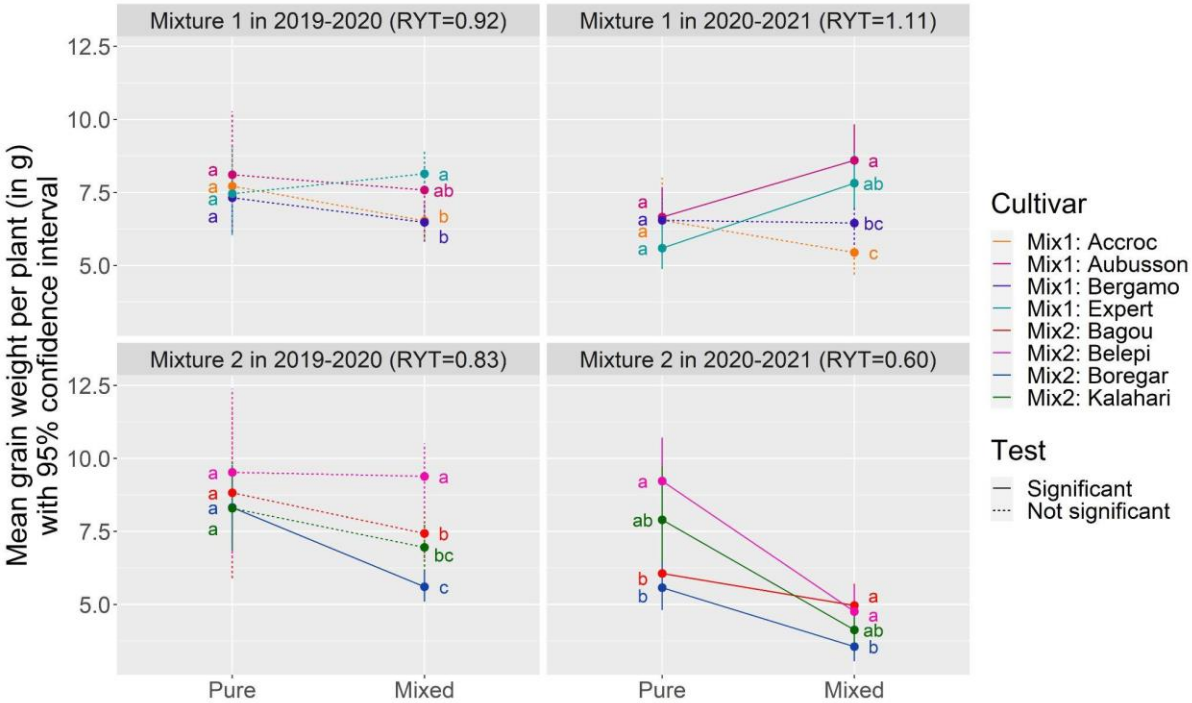


Figure 1: Reaction norms of mean grain weight per plant between pure and mixed stands per cultivar for both mixtures in both seasons. Vertical segments represent confidence intervals at 95%. Letters indicate pairwise differences between cultivars in pure or mixed stands. Line types indicate the statistical significance of the reaction norms.

Dominance relationships among cultivars were defined by testing pairwise yield differences and turning them into rankings. Although trivially done for the pure stands, this was made possible in the mixed stands thanks to our experimental design. For three mixture-season combinations, all cultivars yielded similarly in pure stands but differently in the mixture (Figure 1). In the fourth case (Mixture 1 in 2020-2021), the ranking of one cultivar among the four changed between pure and mixed stands. Nevertheless, overall, neither cultivar rankings nor mean yields were significantly correlated between monoculture and mixture (Figure S4).

Measuring the yield in the mixture per cultivar also allowed us to compute the reaction norms between pure and mixed stands for each cultivar. Out of the sixteen cultivar-season combinations, 7 reaction norms (44%) were significantly different from zero, an indicator of phenotypic plasticity (Figure 1). Among these reaction norms, only two were positive, the ones associated with the overyielding mixture (Mixture 1 in 2020-2021).

The magnitude of yield plasticity in pure versus mixed stand for a given cultivar was regressed on the absolute difference between its monoculture yield and the average monoculture yield of the other cultivars with which it was mixed (Figure 2). The association in the first season was not significant at 5% (slope p-value = 0.35 and $R^2$ = 0.14). The second season however, the association was significant at 5% (slope p-value = 0.046 and $R^2$ = 0.51) with a quantile-quantile plot of residuals being reasonable given the small sample size (Figure S5). The second season, even though a quadratic model could provide a fit visually more appealing, the linear model was retained based on the likelihood ratio test (p-value = 0.21).

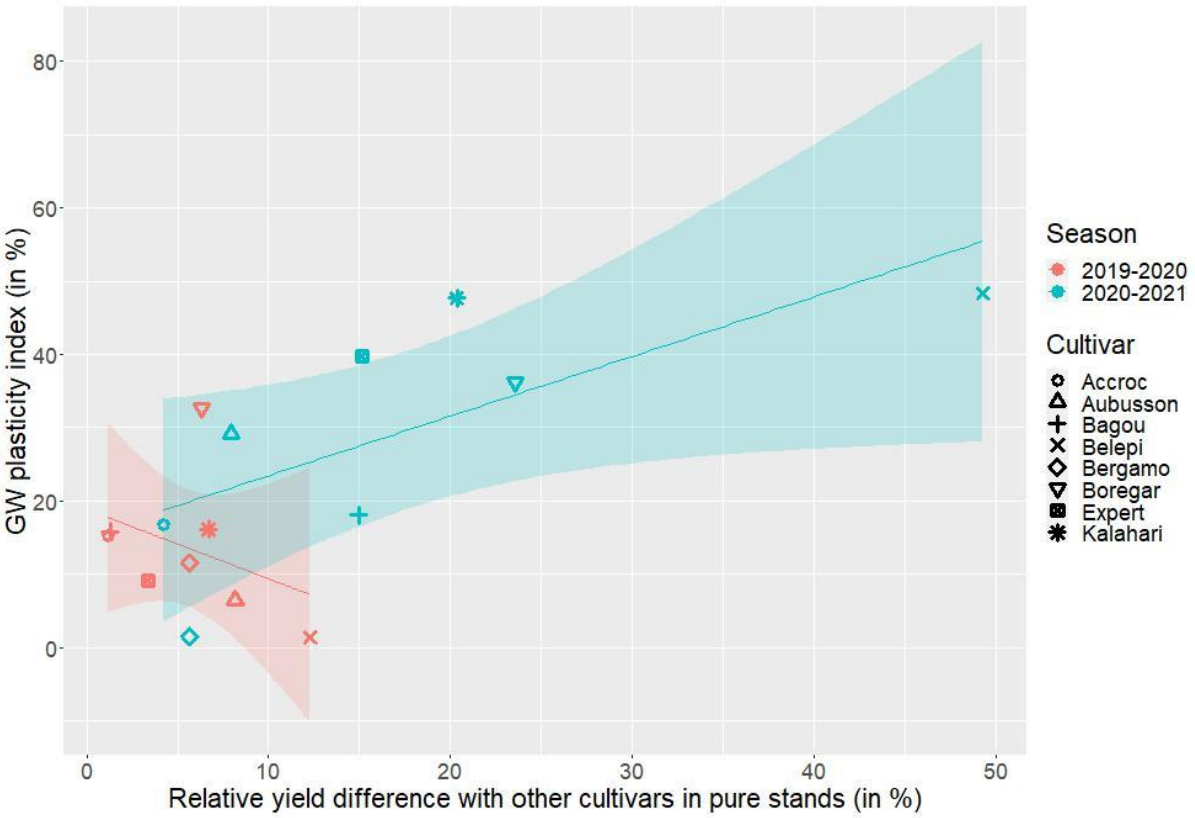

Figure 2: Plasticity between pure and mixed stands for grain weight (GW) per cultivar and season versus the relative difference between the pure-stand grain weight of the focal cultivar and the average of the pure-stand grain weights of the other cultivars in the mixture.

## Plasticity in both tillering emission and regression drives plasticity in spike number which explained most of yield plasticity

Given the cases of significant phenotypic plasticity in grain weight displayed by some cultivars, we decomposed it into the phenotypic plasticity of yield components (Figure 3 for 2019-2020, Figure S6 for 2020-2021). Overall, the plasticity in spike numbers was the main contributor, in five out of seven cases in 2019-2020 (notably for three cultivars in Mixture 2), and also six out of eight cases in 2020-2021. The plasticity in spike number was also significantly associated with the plasticity in harvest index, but with a slope inversion between seasons (Figure S7). Moreover, the plasticities in yield components were strongly correlated, also with a change of sign between seasons (Figure S8).

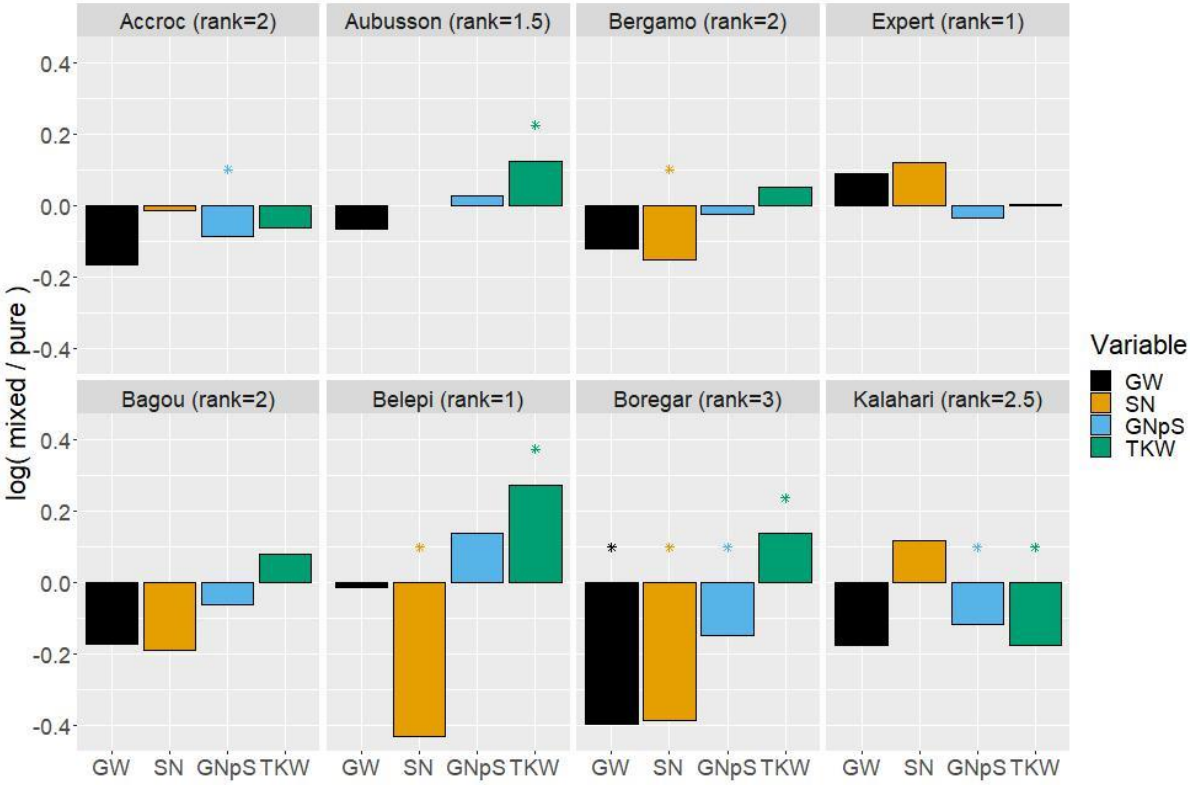


Figure 3: Log values of phenotypic plasticity of grain weight (GW) and its yield components, spike number (SN), grain number per spike (GNpS) and thousand kernel weight (TKW) in 2019-2020. Stars indicate significant differences in pure versus mixed stands for a given variable, genotype and season.

Spike number is the result of tillering, a dynamical process whose particularity is to be non-monotonic. Thanks to our experimental design, we were able to phenotype this process for the first time at the level of individual plants in field conditions for both pure and mixed stands, as exemplified for Mixture 2 in 2019-2020 (Figure 4; see Figure S9 for the other cases).

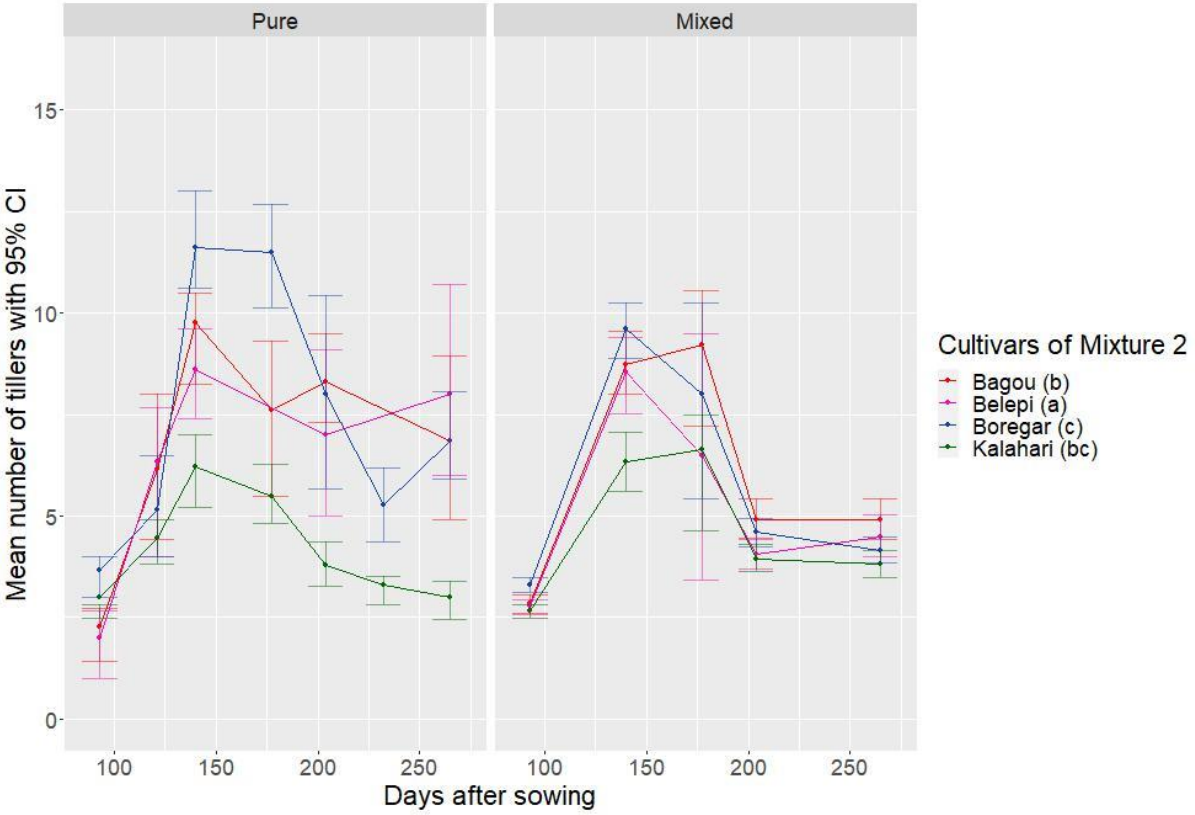


Figure 4: Average tillering dynamics from individual plant data all along the crop cycle for each cultivar of Mixture 2 in 2019-2020 for both pure and mixed stands. Error bars correspond to 95% confidence intervals.

In qualitative terms, the two phases of tillering dynamics, emission and regression separated by a plateau, were clearly visible for both pure and mixed stands. However, these dynamics looked quantitatively different between both stands. Beyond their outcome, spike number, the tillering dynamics themselves were also plastic. Indeed, our two metrics, the maximum number of tillers (MTN) for the emission phase and the number of regressed tillers (NRT) for the regression phase, showed several significant cases of plasticity between stands, as exemplified for Mixture 1 in 2019-2020 (Figure 5; see Figure S10 for the other cases). Furthermore, the plasticities of these metrics could compensate for each other (Figure 5), sometimes completely, hence leading to no plasticity in the spike number, e.g., Accroc in 2019-2020, or only partially hence leading to spike number plasticity, e.g., Bergamo in 2019-2020.

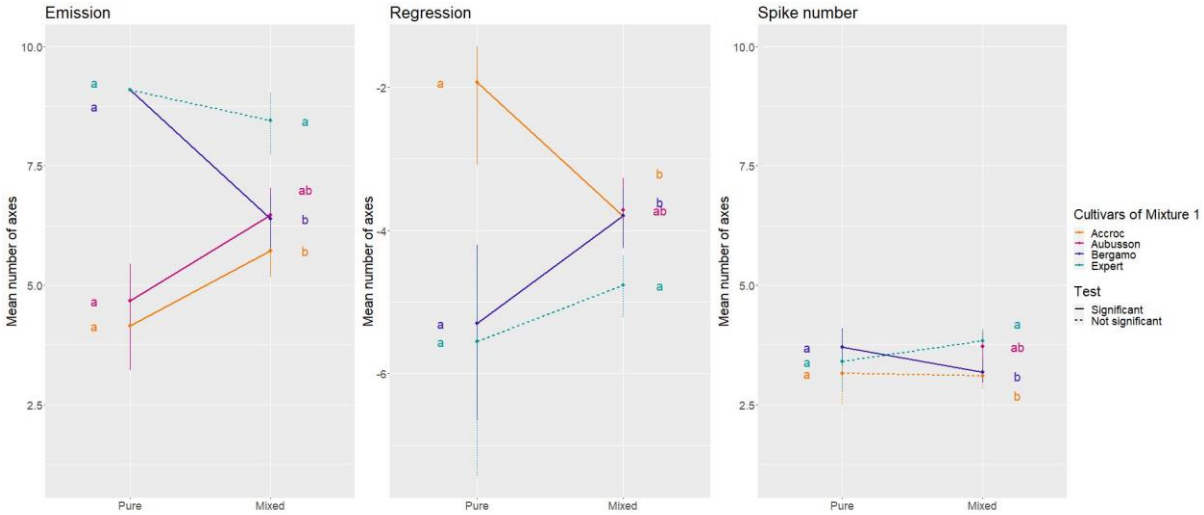


Figure 5: Reaction norms of number of axes per plant during tiller emission (MTN) and regression (-NRT) between pure and mixed stands per cultivar for Mixture 1 in 2019-2020. Vertical segments represent confidence intervals at 95%. Letters indicate pairwise differences between cultivars in pure or mixed stands. Line types indicate the statistical significance of the reaction norms.

## Dynamics of height differentials explain tillering plasticity

Knowing that tillering dynamics are influenced by the perception of a light-quality signal, we monitored the height dynamics of individual plants, testing for phenotypic plasticity between pure and mixed stands at each time point (Figure 6 and Figure S11). Overall, reaction norms with a slope significantly different than zero were observed in all mixture-season combinations and at various dates along the crop cycle.

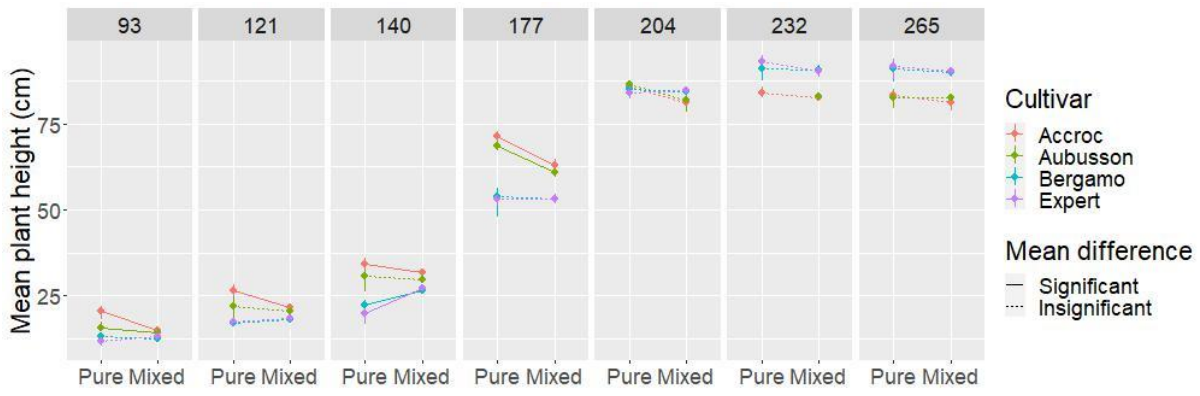


Figure 6: Reaction norms of plant height all along the crop cycle between pure and mixed stands per cultivar for Mixture 1 in 2019-2020. Line types indicate the statistical significance of the reaction norms.

In Mixture 1 in 2019-2020, the two cultivars chosen for their earliness, Accroc and Aubusson, were indeed taller than their neighbors, Bergamo and Expert, early in the crop cycle, particularly between 121 and 177 days after sowing (DAS), because the elongation of their main stem started earlier. This was associated with significant phenotypic plasticity, negative for the early cultivars and positive for the late ones, e.g., at 140 DAS. In 2020-2021, no difference in earliness was observed between cultivars of Mixture 1, yet, several similar cases of phenotypic plasticity were significant. For instance, Bergamo, taller than the others at 177 DAS in pure stands, had a negative reaction norm; on the opposite, Accroc, shorter than the others at 232 DAS, had a positive reaction norm. This pattern was also observed in Mixture 2: in 2019-2020 for Belepi at 121 DAS, Boregar at 177 and 204 DAS; in 2020-2021 for Belepi at 76 DAS, Boregar at 148 DAS and Bagou at 232 DAS.

Moreover, during the phase of tiller emission, the height differentials per cultivar (in comparison with the others) in the mixtures were significantly associated with their plasticity in maximum tiller number (Figure 7). The Pearson correlation coefficient was positive for both seasons: 0.66 [0.36, 0.83] in 2019-2020 (p-value $2.10^{-4}$) and 0.41 [0.08, 0.67] in 2020-2021 (p-

value 0.0186). We also tested this relation during the phase of tiller regression (Figure S12) also controlling for the plasticity during the phase of tiller emission. In 2019-2020 the plasticity in tiller regression was explained only by the plasticity of tiller emission. In 2020-2021 however, the plasticity in tiller regression was significantly associated with both height differential and plasticity on tiller emission.

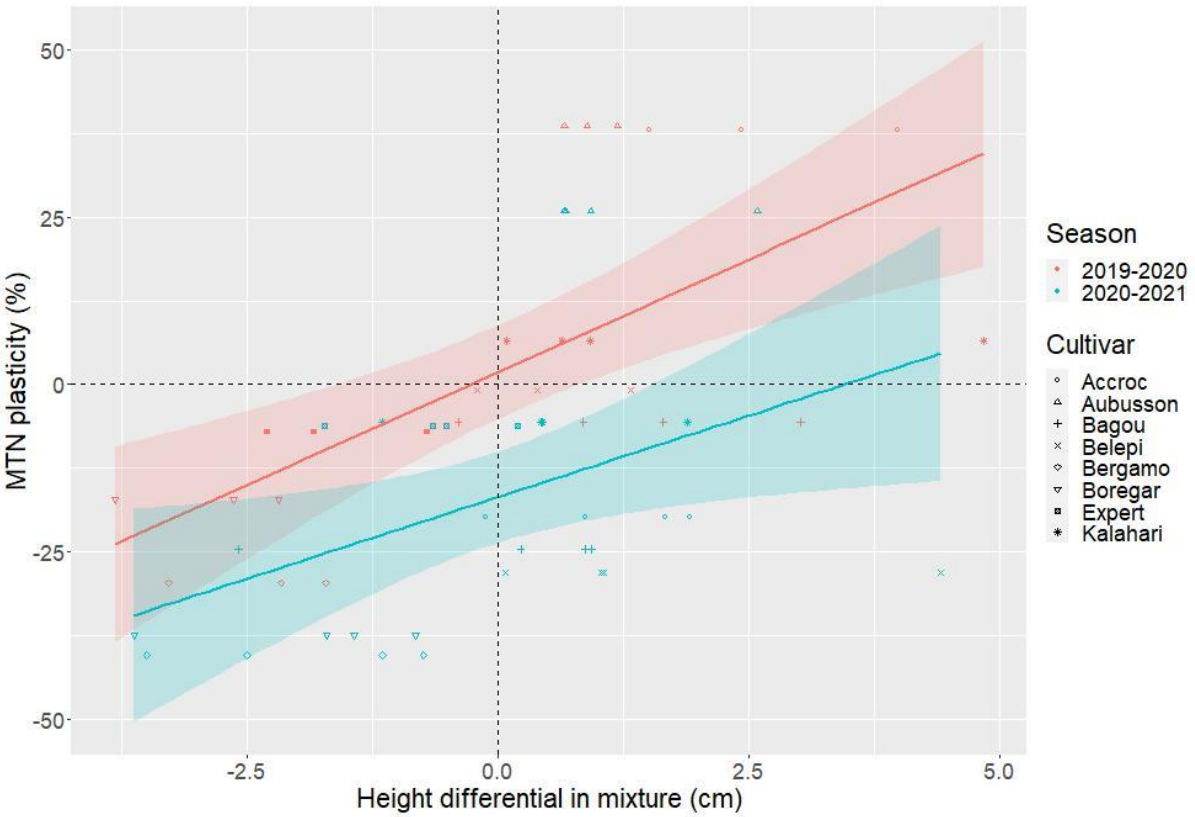


Figure 7: Plasticity of the maximum tiller number versus height differentials in mixture computed during the tiller emission phase.

# Discussion

We proposed a new experimental design and analytical framework to study, in field trials, the reaction norms of cereals in pure versus mixed stands. We applied it to the development and productivity of eight bread wheat genotypes, in pure stand as well as mixed into two four-way mixtures, over two seasons.

The yield comparison between pure and mixed stands displayed a large variation. Only one favorable mixture over four was observed, contradicting the usual beneficial performance of mixtures. In agreement with our first hypothesis, that variation was due to the phenotypic plasticity of cultivars, with both positive and negative reaction norms from pure to mixed stand that affected the dominance relationships between cultivars. The results also partially confirmed our second hypothesis: the second season, the magnitude of yield plasticity for a given cultivar was significantly positively associated with the difference of its yield with the three other cultivars, observed in pure stand. We then showed that yield plasticity resulted from yield-component plasticities, and confirmed our third hypothesis of a main contribution of spike number plasticity. Plasticity in spike number was itself driven by plasticity in both the tiller emission and regression phases that, importantly, could compensate for each other. Plasticity in the dynamics of plant height, most notably during the bolting phase, was identified and displayed a convergence pattern: taller (respectively, smaller) cultivars in a pure stand were smaller (resp. taller) in a mixed stand. The height differentials of cultivars in mixture were also significantly associated with the tillering plasticities. Furthermore, we demonstrated that yield-component plasticities were strongly correlated, and that plasticity in spike number could be canceled or amplified by the plasticity of other components. The plasticity in spike number was also significantly associated with the plasticity in harvest index. Finally, our results highlighted

the importance of the environmental conditions, with several relationships changing sign from one season to the next.

Overall our results showed that the most integrative trait of interest, yield, can be stabilized in mixture over contrasted seasons, as well as its main component, spike number. Yet the underlying determinants of spike number, tiller emission and regression, can be highly variable. The stability in spike number, and hence grain weight, arises through the net result of cancelation-amplification of the underlying determinants as well as of the other yield components, grain number per spike and thousand kernel weight.

## Plasticity in tillering

In general, phenotypic plasticity of a given trait can be classified as advantageous, neutral or disadvantageous depending on the relationships with fitness (Alpert and Simms, 2002). Moreover, the plasticity in a given trait can be qualified as *active* in the sense of "anticipatory, and often highly integrated, phenotypic changes in response to some environmental cue or signal", whereas the plasticity in another trait would be best viewed as *passive*, i.e., as a "consequence of the environment, such as stunted growth owing to low resource levels" (Forsman, 2015).

Given the early detection of light signals (R:FR), their integration as a bet on future competition and the consequences in terms of architectural changes, plasticity in tillering can be characterized as active. As the outcome for a plant of an early bet, here on tillering, was more consequential on yield than would be a late bet, such as on thousand kernel weight, improving the odds of the bet on tillering would justify maintaining the capacity to detect variation in light cues and respond appropriately to them. Moreover, we observed a hierarchy of yield-component plasticities in pure versus mixed stands in agreement with known results in the literature obtained when comparing stress versus no-stress conditions, (Sadras and Slafer,

2012). In the majority of cases, the plasticity in spike number was higher than the plasticities of the grains per spike and thousand kernel weight. The plasticity hierarchy in yield components can hence be interpreted as reflecting differences between active and passive plasticity, as well as the contrasted costs associated with these plasticities. Because of the processes involved in responding to specific signals, active plasticity can come at a cost (DeWitt et al., 1998). Due to the sequential building of yield components in annual plants, we can further hypothesize that the tradeoff between the cost of active plasticity and its advantages will be more beneficial for early processes than late ones, as illustrated by the tillering dynamics and the R:FR signaling.

We also observed phenotypic plasticity in height during plant growth and it displayed a convergence pattern consistent with the shade avoidance syndrome, as already documented in barley varietal mixtures (Dahlin et al, 2019). Because this syndrome is associated with more biomass being allocated to vegetative structure to the detriment of reproductive ones, such a plasticity in height is considered harmful in crops, with ongoing efforts to reduce it in wheat (Wille et al., 2017). A reduced plasticity in stem elongation was for instance observed in modern, smaller cultivars in comparison to older, taller ones for oat (Semchenko and Zobel, 2005) and wheat (Weiss et al., 2009). Interestingly, we observed a similar convergence pattern in terms of tillering dynamics (Figure 4) as well as a significant relationship between the plasticity in tiller emission and height differential in mixture (Figure 7). This possibly reflects a causal interplay between height and tillering due to a shared mechanism, as exemplified by the pleiotropic effect of Rht genes (Xu et al., 2023).

## Significance for assembly rules and breeding strategies

Regarding assembly rules, farmers often avoid mixing cultivars of different height or earliness (Borg et al., 2018). However, our results show that the tallest cultivars at maturity or the earliest

ones are not always dominating in the mixture. For instance, Kalahari had the highest final height in Mixture 2 but it did not dominate whatever the season. Similarly, Accroc and Aubusson were the earliest cultivars in Mixture 1, especially the first season, but their dominance ranks this season were respectively second and third. Phenotypic data such as final height and heading date, easily recorded in monoculture trials and largely made available to farmers, may hence not be that informative to predict the outcome of competition in varietal mixtures.

Furthermore, our study highlights the importance of tillering and its plasticity to decipher the behavior of cultivars in mixtures and the productivity of mixtures in general. The observed patterns of plasticity in both tillering phases notably depended on the season (genotype-by-genotype-by-season interactions, GxGxE), which complicates the definition of mixture assembly rules. Hence, knowing the yield variability of cultivars in monoculture over various environments, although useful (e.g., see Wuest et al, 2021), may not be sufficient. For instance, the grain yield of cv. Belepi in pure stand is the same for both seasons, but it differs significantly in mixture (similarly for Kalahari). Complex interactions between plants in mixture and climate make mixture performances hard to predict. The plant-plant interactions in mixtures (GxG) may strongly depend on abiotic conditions (GxGxE), which leads to complex interactions hard to disentangle.

Although it may be relevant to select against plasticity in height due to the shade avoidance syndrome (e.g., Wille et al., 2017), such a strategy may ultimately be counterproductive if plasticities in height and tillering have partially shared genetic architecture. Barot et al. (2017) suggested that plasticity in some traits may be worth keeping: tillering may be one such example.

To breed for mixture yield, Litrico and Violle (2015) proposed to maximize the phenotypic variance in agronomic traits between mixture components, while minimizing it for interaction

traits. They defined agronomic traits as the ones at least partially determining agronomic value (e.g., grain yield), and interaction traits as those involved in interactions with neighbors. As our study demonstrates, tillering fits both these definitions, hence questioning the applicability of this framework.

## Perspectives and open questions

Because of the detailed monitoring of individual plants in mixed stands, it was feasible to experiment with only eight cultivars and two quaternary mixtures. Despite the small number of mixtures, the observed RYTs were representative of the variability in the literature (Borg et al., 2018). Binary mixtures are expected to ease the study of interactions, at the cost of restricting the number of observed varieties. We made the choice to experiment with mixtures more complex than binary mixtures, to stick to the reality of farmers' practices, but with more complex interactions to disentangle. Furthermore, similarly to other studies (e.g., Dahlin et al., 2020), we ignored the below-ground organs, even though root traits can contribute to a functional understanding of mixing effects (Montazeaud et al., 2018). However, adding this layer of phenotyping to our experimental design would have required the usage of a field-based, non-destructive root phenotyping protocol which remains to be developed. Our analysis of reaction norms for yield and how they are explained by phenotypic traits such as height and earliness is strongly based on ecophysiological knowledge. Although plant-plant interactions consist in part in trophic interactions, i.e., competition for resources (Grace and Tilman, 1990), signals can play a major role on phenotypic plasticity, as illustrated for tillering by the R:FR sensing. More and more signals involved in plant-plant communication are described (see Subrahmaniam et al., 2018 and Ninkovic et al., 2019 for reviews), and they certainly deserve better attention to understand the outcomes of plant interactions in mixtures.

Predicting mixture productivities will require a better knowledge of plant development in both pure and mixed stands, notably regarding the tillering process. Due to the phenotypic plasticity between stands, the genotype-by-genotype interactions, and their dependency on environmental conditions, observing mixtures will not be dispensable. From our results, a challenge would consist in phenotyping at high throughput the maximum number of tillers at stage BBCH30, possibly at several densities. In monoculture a UAV-based strategy seems feasible (Wu et al., 2022), but transposing it in mixture at large scale will require the development of a precision sower. Another strategy is to develop functional-structural plant models with an explicit tillering process (e.g., Blanc et al., 2021), allowing an *in-silico* exploration of mixture behavior after calibration on data as the ones we produced here. Combining both strategies, i.e., UAV-based phenotyping and process-based modeling, is already feasible for wheat in pure stands (Jégo et al., 2012; Yang et al., 2021), but remains to be explored in mixed stands.

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# Supplementary Material

## Table S1

| Mixture | Cultivar | Maintainer (registration year, country) | Heading (DOY) | Final height (cm) | Awns |
|---|---|---|---|---|---|
| **Mixture 1** | Accroc | RAGT (2010, FR) | 128.4 | 87.02 | Yes |
| | Aubusson | Limagrain (2001, FR) | 129.9 | 87.03 | No |
| | Bergamo | RAGT (2012, FR) | 145.3 | 92.17 | No |
| | Expert | Syngenta (2007, FR) | 144.1 | 90.93 | No |
| **Mixture 2** | Bagou | Asur Plant Breeding (2007, FR) | 136.7 | 82.97 | No |
| | Belepi | Lemaire Deffontaines (2013, FR) | 138.1 | 100.93 | No |
| | Boregar | RAGT (2008, FR) | 140.7 | 84.08 | Yes |
| | Kalahari | Limagrain (2010, DE) | 141.9 | 115.19 | No |

Table S1 - Composition of both mixtures, as well as heading date (since January 1) and height at maturity of the cultivars (source: BreedWheat project).

Table S2

| Mixture | Season | Cultivar | Letters from the Tukey tests | | Dominance rank in mixed stands | Dominance status in mixed stands |
|---|---|---|---|---|---|---|
| | | | pure | mixed | | |
| Mixture 1 | 2019-2020 | Accroc | a | b | 2 | dominated |
| | | Aubusson | a | ab | 1.5 | dominating |
| | | Bergamo | a | b | 2 | dominated |
| | | Expert | a | a | 1 | dominating |
| | 2020-2021 | Accroc | a | c | 3 | dominated |
| | | Aubusson | a | a | 1 | dominating |
| | | Bergamo | a | bc | 2.5 | dominated |
| | | Expert | a | ab | 1.5 | dominating |
| Mixture 2 | 2019-2020 | Bagou | a | b | 2 | dominating |
| | | Belepi | a | a | 1 | dominating |
| | | Boregar | a | c | 3 | dominated |
| | | Kalahari | a | bc | 2.5 | dominated |
| | 2020-2021 | Bagou | b | a | 1 | dominating |
| | | Belepi | a | a | 1 | dominating |
| | | Boregar | b | b | 2 | dominated |
| | | Kalahari | ab | ab | 1.5 | ~dominated |

Table S2 - Table with Tukey classes and matching dominance ranks for each genotype over the two growing seasons.

# Figure S1

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | Ber | Aub | Acc | Ber | Exp | Acc | Aub | Exp | Aub | Ber | Ber | Exp | 22 |
| D | Exp | Exp | Acc | Aub | Aub | Exp | Ber | Acc | Exp | Aub | Acc | Exp | 21 |
| E | Acc | Aub | Exp | Exp | Ber | Acc | Aub | Aub | Ber | Aub | Exp | Ber | 20 |
| F | Exp | Acc | Ber | Aub | Aub | Exp | Acc | Ber | Exp | Acc | Aub | Acc | 19 |
| G | Acc | Acc | Aub | Exp | Acc | Ber | Exp | Aub | Acc | Exp | Ber | Exp | 18 |
| H | Exp | Ber | Ber | Aub | Aub | Acc | Exp | Ber | Acc | Exp | Acc | Aub | 17 |
| A | Aub | Acc | Exp | Acc | Ber | Exp | Aub | Acc | Aub | Acc | Ber | Exp | 16 |
| B | Ber | Acc | Acc | Exp | Ber | Acc | Aub | Exp | Ber | Acc | Aub | Ber | 15 |
| C | Aub | Exp | Ber | Aub | Aub | Ber | Ber | Aub | Aub | Ber | Aub | Exp | 14 |
| D | Ber | Aub | Exp | Acc | Exp | Aub | Ber | Ber | Exp | Exp | Acc | Ber | 13 |
| E | Acc | Acc | Exp | Acc | Ber | Acc | Acc | Aub | Aub | Acc | Aub | Exp | 12 |
| F | Exp | Aub | Ber | Aub | Aub | Exp | Ber | Acc | Exp | Exp | Acc | Ber | 11 |
| G | Ber | Acc | Acc | Acc | Exp | Acc | Aub | Acc | Ber | Aub | Ber | Exp | 10 |
| H | Exp | Acc | Ber | Aub | Ber | Exp | Ber | Ber | Aub | Aub | Acc | Ber | 9 |
| A | Aub | Exp | Exp | Acc | Acc | Exp | Aub | Exp | Acc | Ber | Aub | Exp | 8 |
| B | Ber | Ber | Aub | Ber | Aub | Ber | Acc | Acc | Exp | Aub | Acc | Ber | 7 |
| C | Acc | Acc | Exp | Acc | Acc | Exp | Ber | Exp | Ber | Ber | Acc | Aub | 6 |
| D | Ber | Exp | Ber | Aub | Exp | Aub | Aub | Acc | Aub | Ber | Ber | Exp | 5 |
| E | Aub | Acc | Aub | Aub | Ber | Acc | Ber | Acc | Exp | Acc | Aub | Exp | 4 |
| F | Acc | Ber | Exp | Acc | Exp | Aub | Acc | Ber | Exp | Aub | Ber | Exp | 3 |
| G | Exp | Aub | Aub | Acc | Aub | Ber | Exp | Aub | Acc | Exp | Acc | Ber | 2 |
| H | Acc | Exp | Ber | Aub | Exp | Aub | Acc | Ber | Aub | Ber | Ber | Exp | 1 |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |

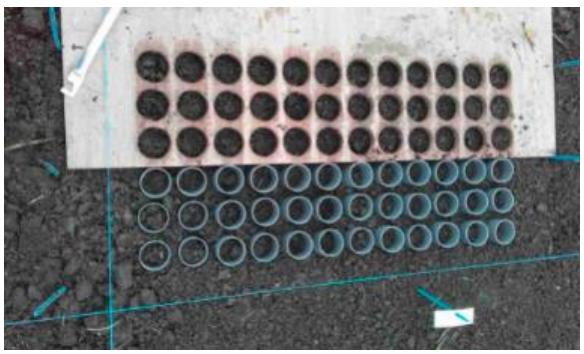
A) Mixture 1

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | Bel | Bag | Bel | Bag | Bag | Bor | Bag | Bor | Kal | Bel | Bor | Bor | 22 |
| D | Bor | Bel | Kal | Bor | Bel | Bel | Kal | Kal | Bel | Bag | Bor | Kal | 21 |
| E | Bag | Bor | Bel | Bag | Kal | Kal | Bor | Bag | Kal | Kal | Bel | Bel | 20 |
| F | Bel | Kal | Bel | Kal | Bor | Bag | Kal | Kal | Bel | Bor | Kal | Bag | 19 |
| G | Bag | Bag | Bel | Kal | Bor | Kal | Bag | Bor | Bag | Kal | Bel | Bor | 18 |
| H | Kal | Kal | Bor | Bag | Bag | Bag | Bel | Kal | Bag | Bor | Bag | Kal | 17 |
| A | Bor | Bag | Kal | Bel | Bor | Kal | Bor | Bag | Kal | Bel | Bel | Bag | 16 |
| B | Bel | Bel | Bor | Bor | Bag | Bel | Kal | Bel | Bor | Bag | Bag | Bel | 15 |
| C | Bel | Kal | Bel | Bor | Kal | Bel | Bor | Bor | Bel | Bel | Bor | Bor | 14 |
| D | Bor | Bag | Kal | Bag | Bor | Bel | Bag | Bel | Bag | Kal | Bel | Bel | 13 |
| E | Bag | Kal | Bor | Bel | Bel | Kal | Bag | Bor | Bag | Kal | Kal | Bag | 12 |
| F | Bel | Kal | Bel | Bor | Bag | Bor | Bel | Kal | Bag | Bag | Bel | Bor | 11 |
| G | Bag | Bor | Kal | Bor | Bor | Bel | Bel | Kal | Bel | Bor | Bor | Bag | 10 |
| H | Bor | Bel | Bor | Bel | Bag | Kal | Bel | Bag | Bor | Kal | Kal | Bag | 9 |
| A | Bel | Bag | Kal | Bag | Bel | Kal | Bor | Kal | Bor | Bel | Bag | Kal | 8 |
| B | Bor | Bor | Bag | Bor | Kal | Bor | Bag | Bag | Bel | Bel | Bor | Bel | 7 |
| C | Kal | Bor | Kal | Bel | Bag | Bor | Kal | Bel | Bor | Bag | Bag | Kal | 6 |
| D | Bag | Bel | Bag | Bor | Kal | Bel | Bor | Kal | Bor | Bag | Kal | Bel | 5 |
| E | Bor | Kal | Bel | Bag | Bel | Kal | Bel | Kal | Bel | Kal | Kal | Bor | 4 |
| F | Kal | Bag | Bor | Kal | Bor | Bel | Bag | Bag | Bag | Bor | Bag | Bag | 3 |
| G | Kal | Bel | Kal | Bag | Bor | Kal | Bag | Bel | Bor | Bag | Bel | Bag | 2 |
| H | Bag | Bor | Bel | Kal | Bel | Kal | Bag | Kal | Bor | Bag | Kal | Bor | 1 |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |

B) Mixture 2

Figure S1 - Spatial distribution of cultivars in “nano-plots” for each mixture for both years.

# Figure S2



A) At sowing

B) After emergence

Figure S2 - Images of “nano-plots” of mixtures with PVC rings in 2019-2020.

# Figure S3

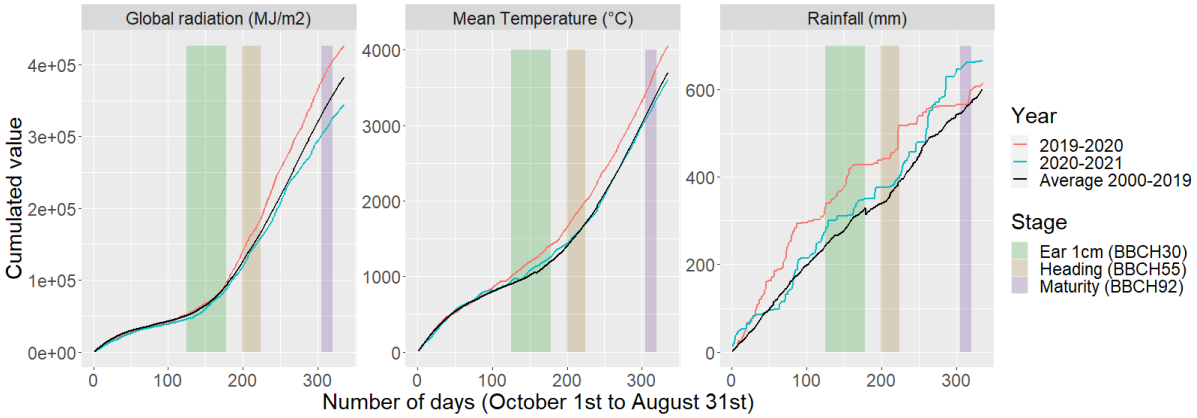
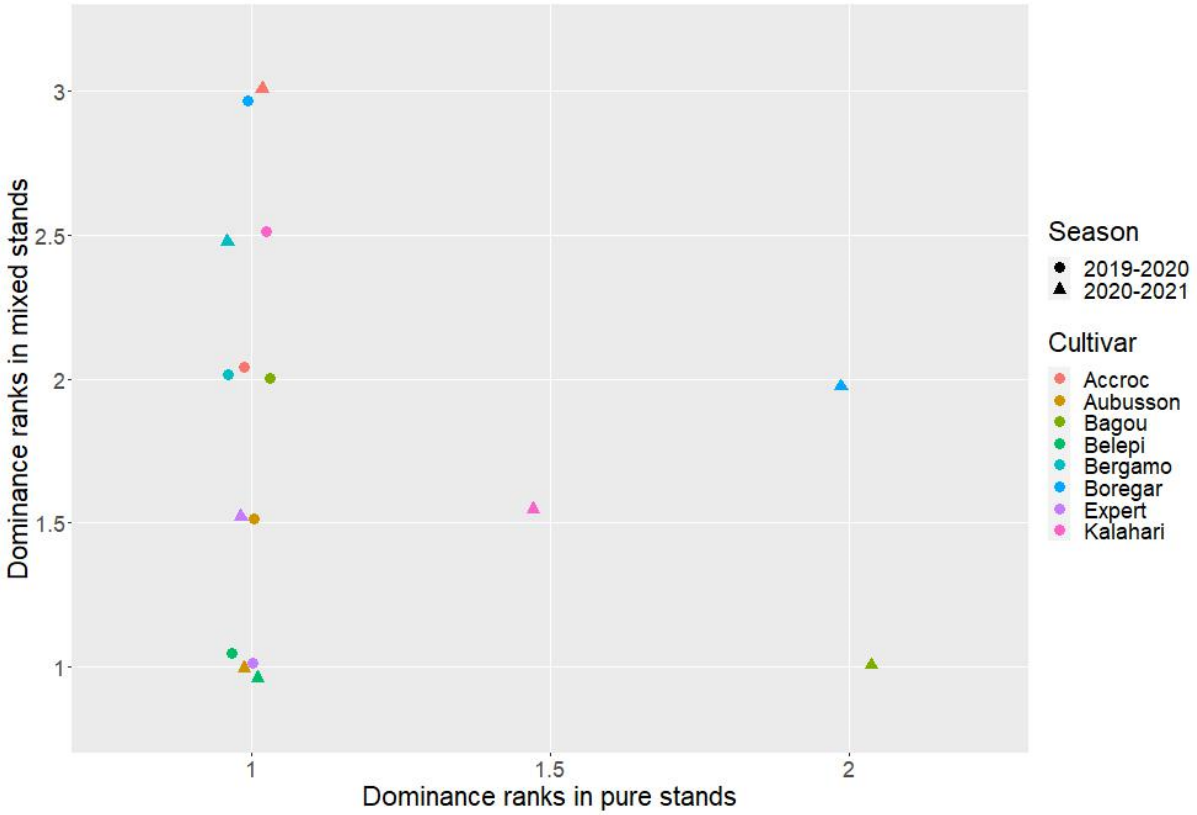
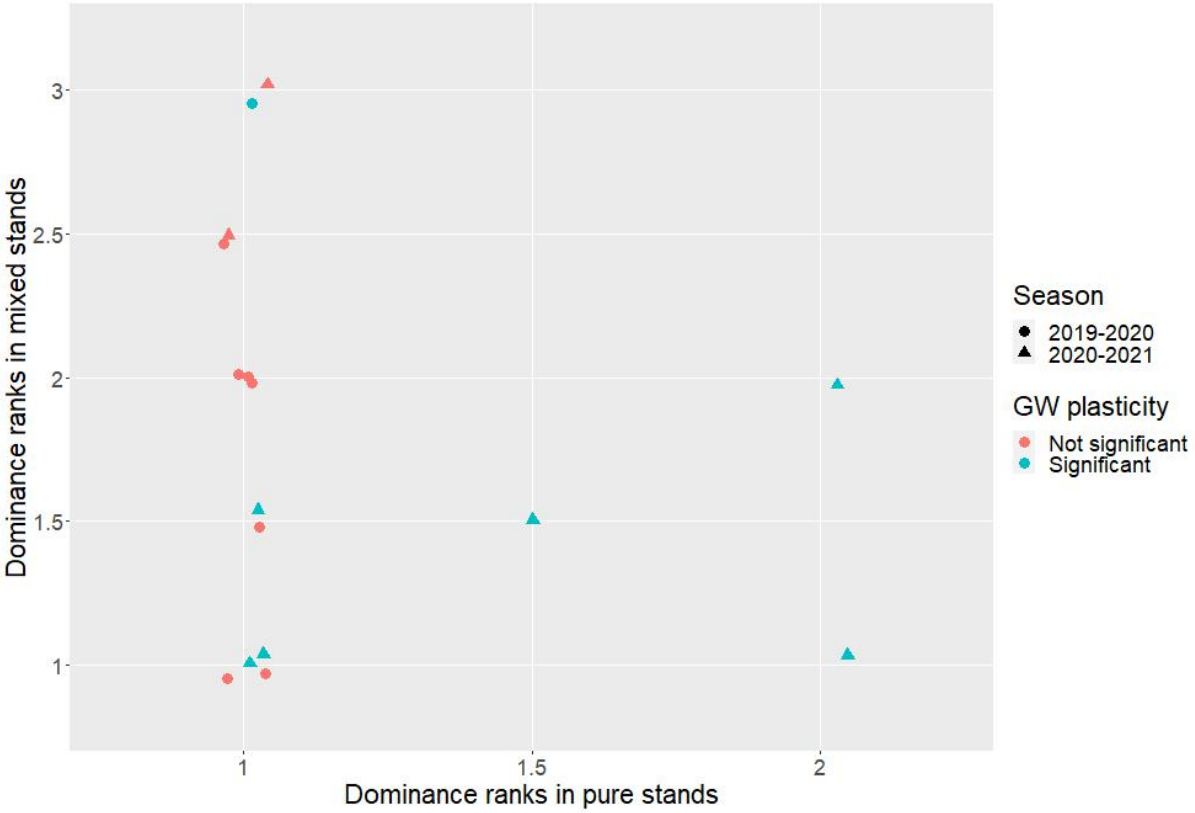



Figure S3 - Cumulative daily global radiation, mean temperature and rainfall for both seasons, in comparison with their respective average from 2000 to 2019. The stages "ear 1cm", "heading" and "maturity" correspond respectively to BBCH30, BBCH55 and BBCH92.

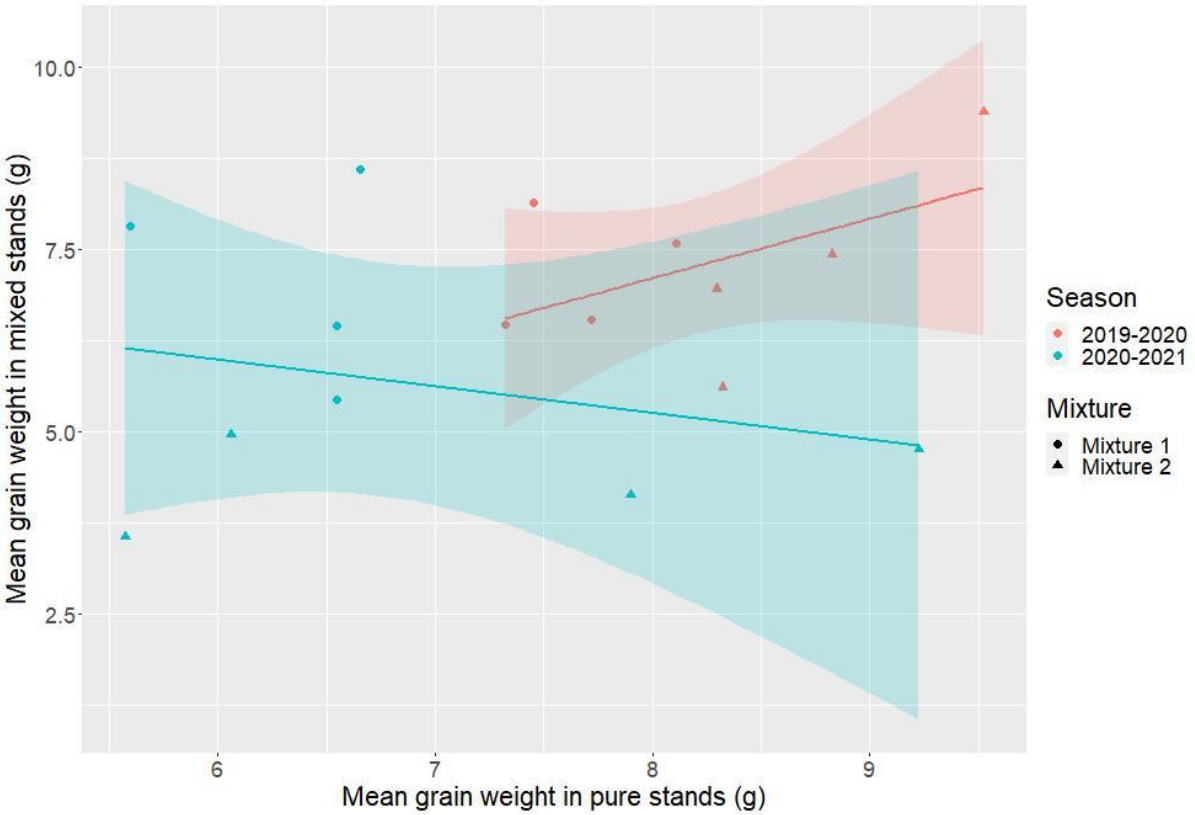
# Figure S4




A) Colour by genotype




B) Colour by plasticity significance



C) Using mean grain weight, Pearson correlations of 0.51 in 2019-2020 (p-value = 0.19) and -0.26 in 2020-2021 (p-value = 0.54)

Figure S4 - Correlation between dominance ranks (A, B; overall Pearson correlation coefficient at -0.19, p-value = 0.48) or mean grain weights (C; overall Pearson correlation coefficient at 0.26, p-value = 0.33) in pure versus mixed stands.

Figure S5

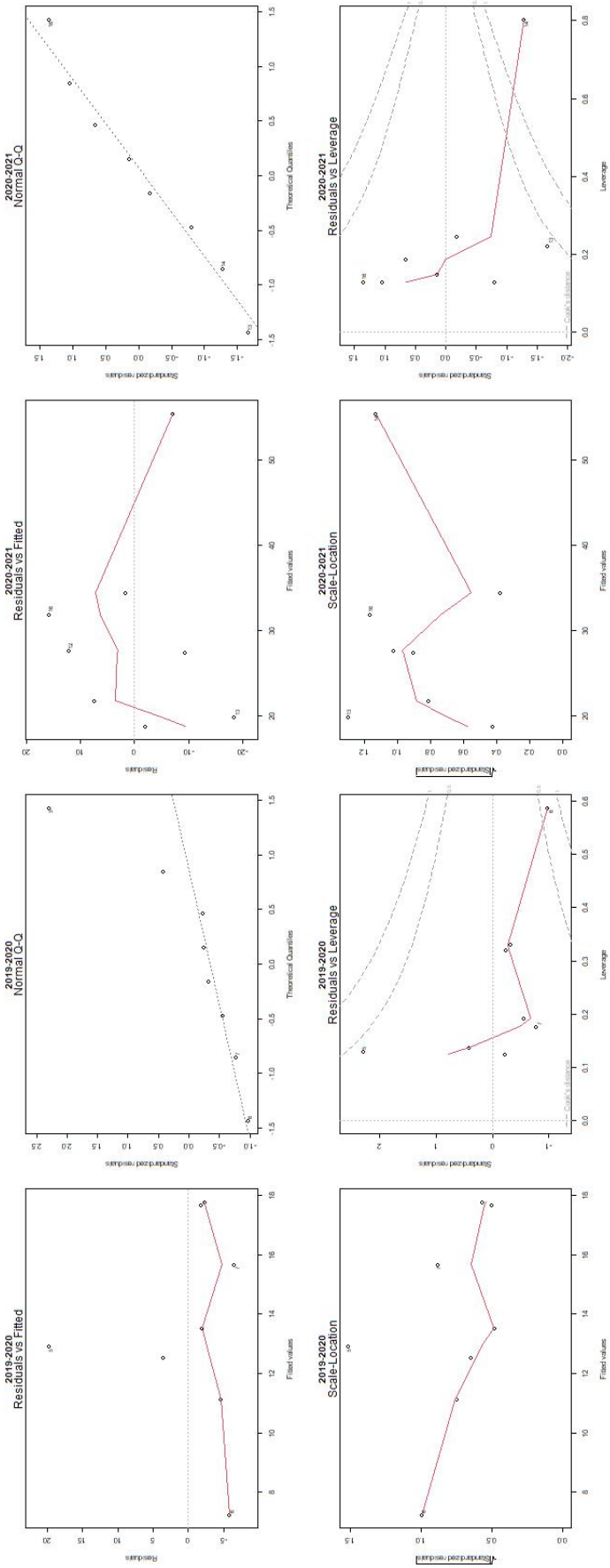

2019-2020
Residuals vs Fitted
Residuals
Fitted values
2019-2020
Normal Q-Q
Standardized residuals
Theoretical Quantiles
2019-2020
Scale-Location
√|Standardized residuals|
Fitted values
2019-2020
Residuals vs Leverage
Standardized residuals
Cook's distance
Leverage
2020-2021
Residuals vs Fitted
Residuals
Fitted values
2020-2021
Normal Q-Q
Standardized residuals
Theoretical Quantiles
2020-2021
Scale-Location
√|Standardized residuals|
Fitted values
2020-2021
Residuals vs Leverage
Standardized residuals
Cook's distance
Leverage

Figure S5: Plot diagnostics of the linear regressions per season in figure 2.

# Figure S6

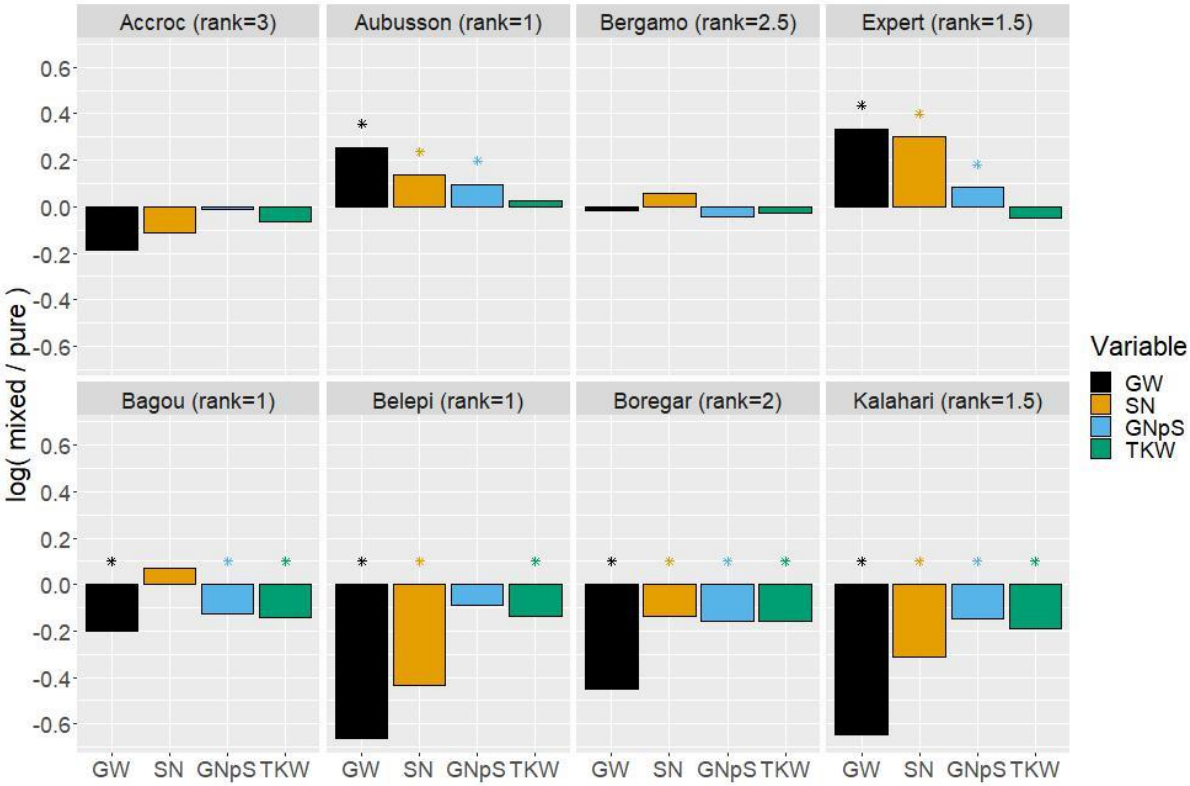



Figure S6: Log values of phenotypic plasticity of grain weight (GW) and its yield components, spike number (SN), grain number per spike (GNpS) and thousand kernel weight (TKW) in 2020-2021. Stars indicate significant differences in pure versus mixed stands for a given variable, genotype and season.

Figure S7

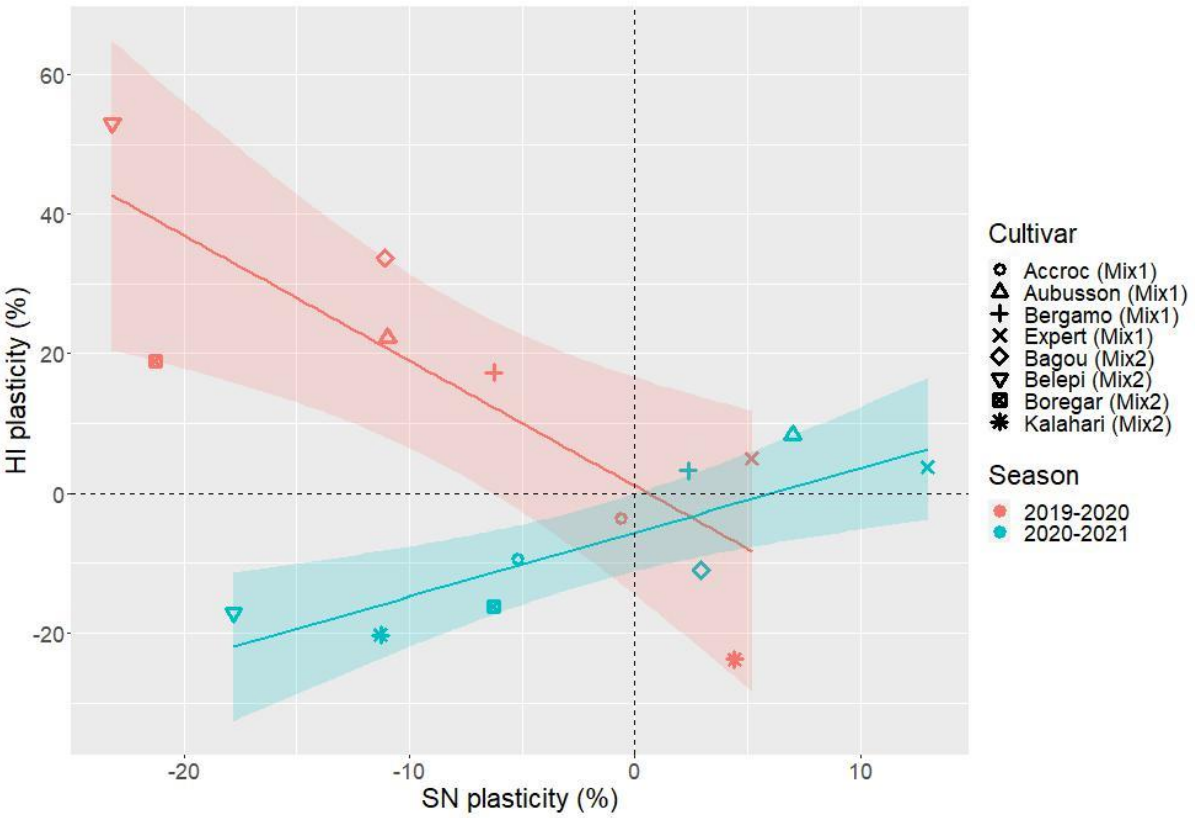
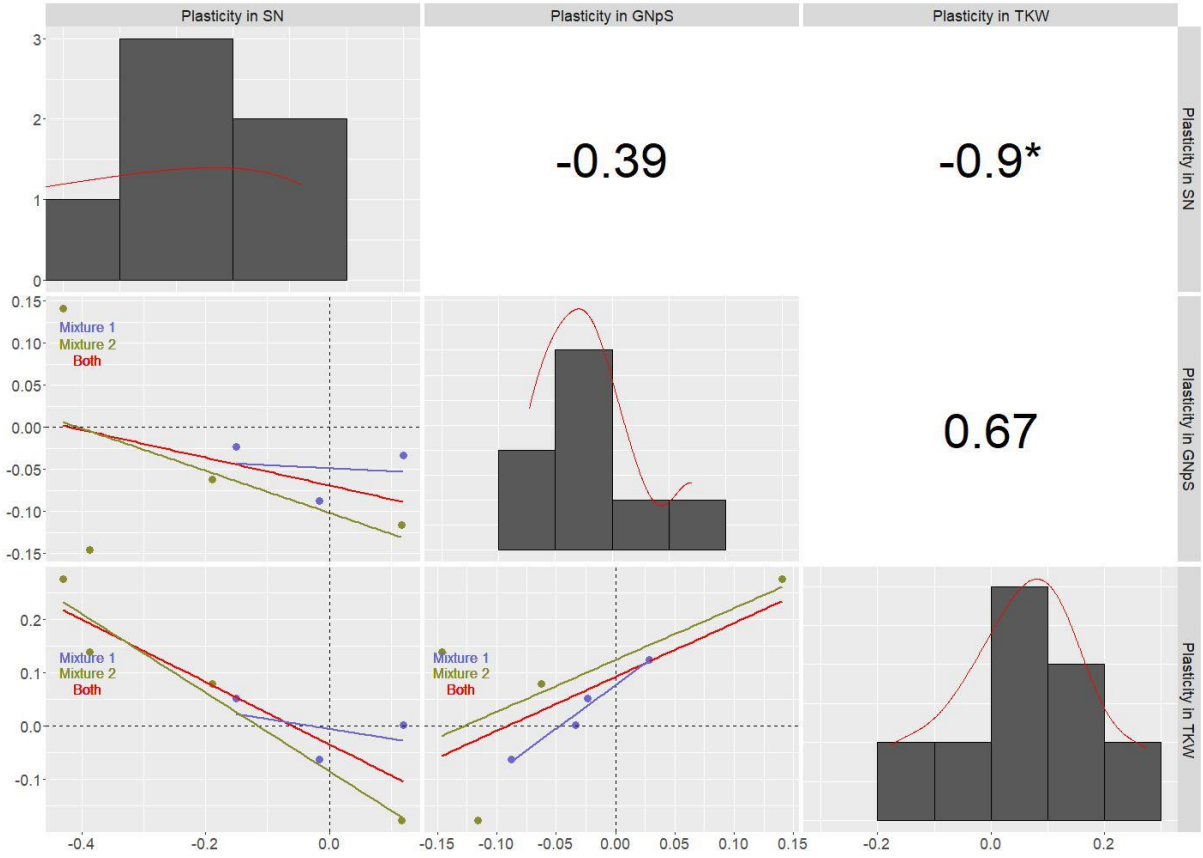
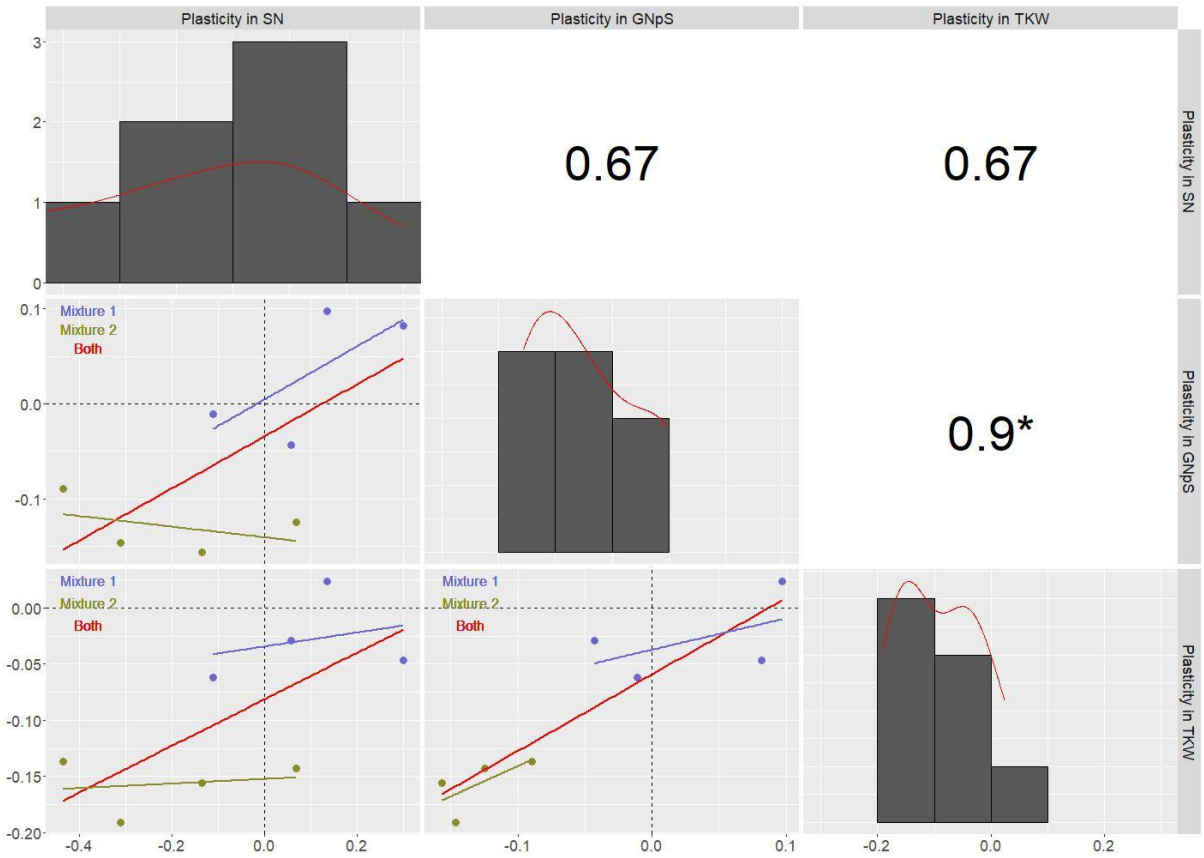



Figure S7: Plasticity between pure and mixed stands in harvest index (HI) versus in spike number (SN) per cultivar and season. A positive value indicates a higher trait value in the mixed stand.

Figure S8



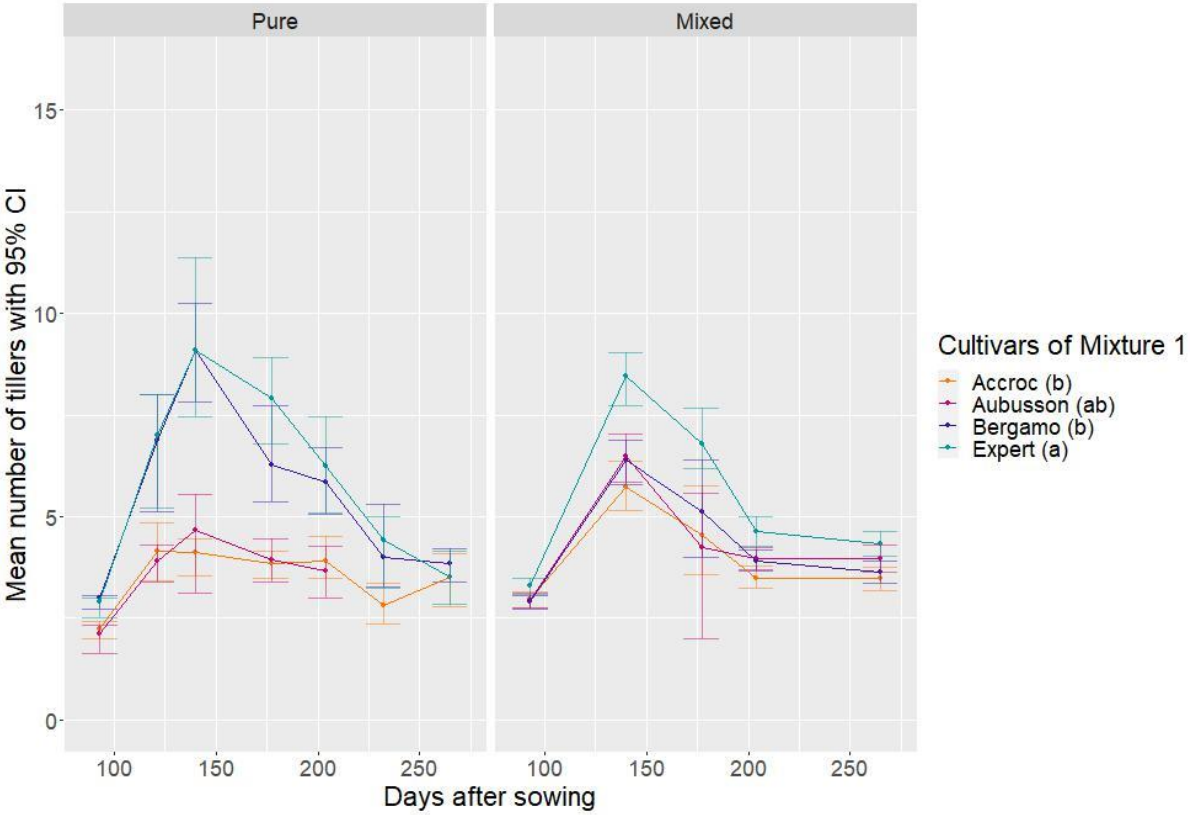

A) 2019-2020



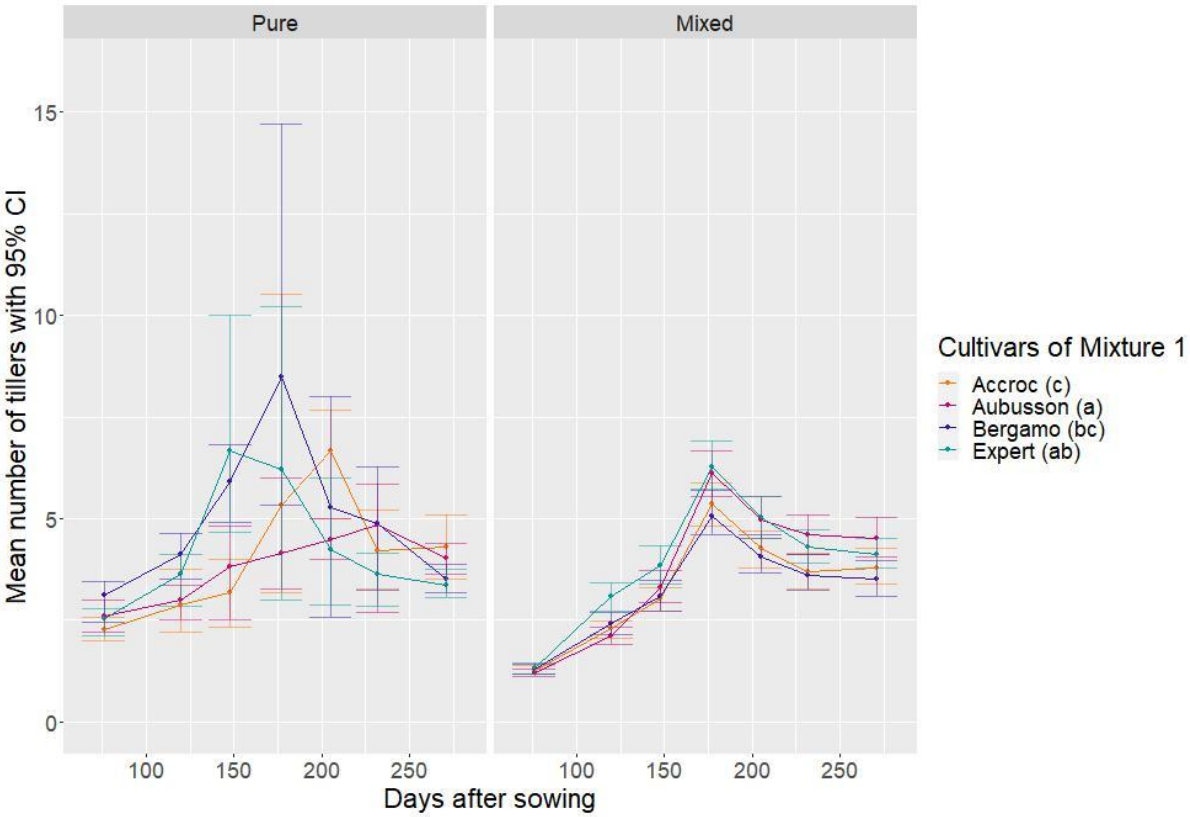

B) 2020-2021

Figure S8: Distributions along the diagonal of the phenotypic plasticity in pure versus mixed stands for grain weight (GW) and its yield components, spike number (SN), grain number per spike (GNpS) and thousand kernel weight (TKW). Pairwise scatterplots are below the diagonal, in green for cultivars of Mixture 1 and purple for cultivars of Mixture 2 with corresponding linear regressions for each mixture and both (in red). Pearson correlations are above the diagonal, with stars to indicate significance at the 5% level.

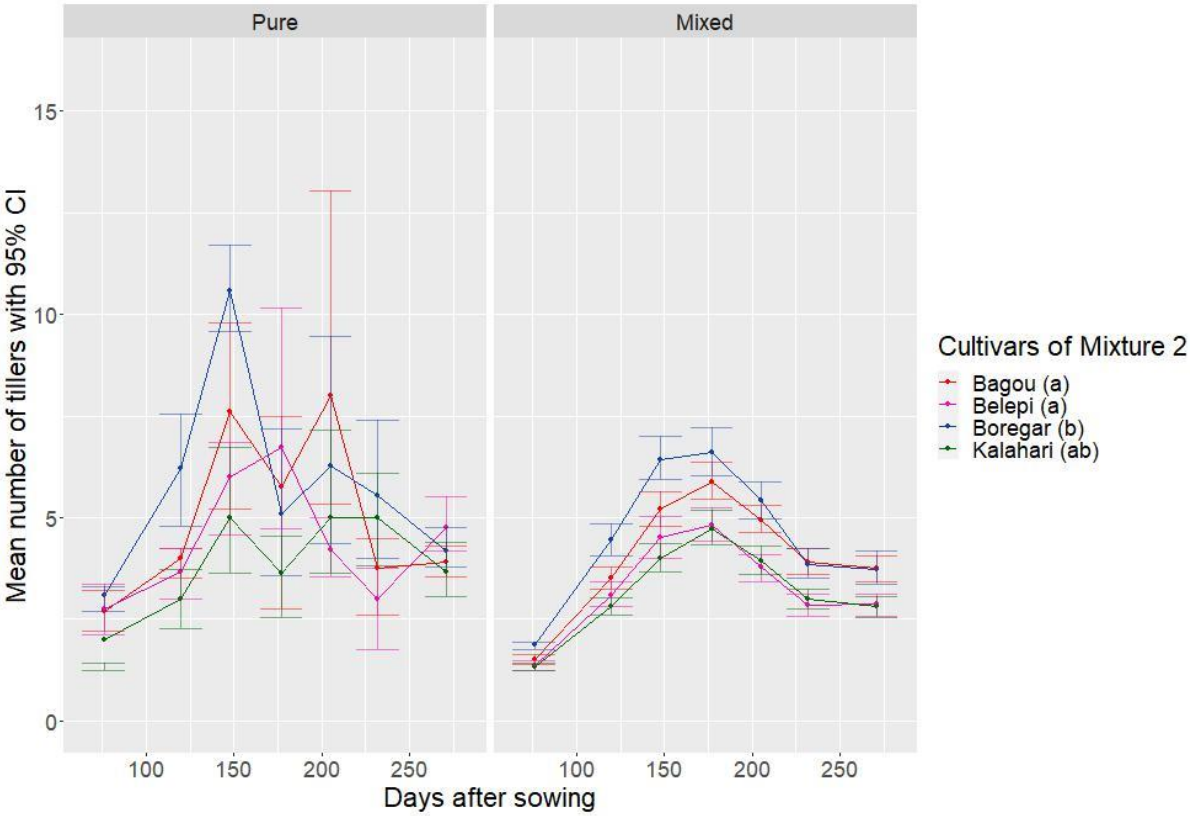
Figure S9




A) Mixture 1 in 2019-2020



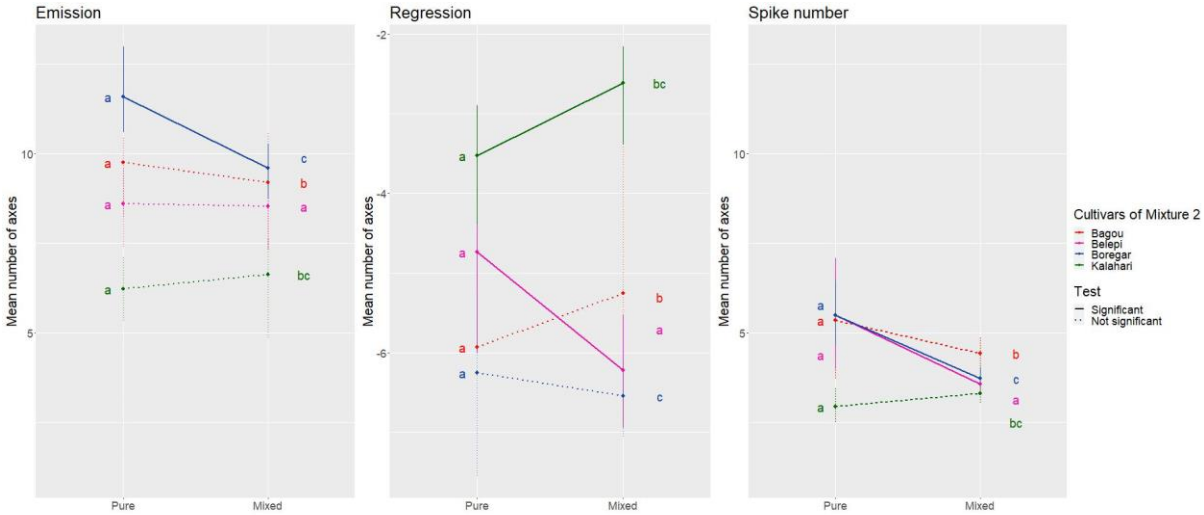
B) Mixture 1 in 2020-2021



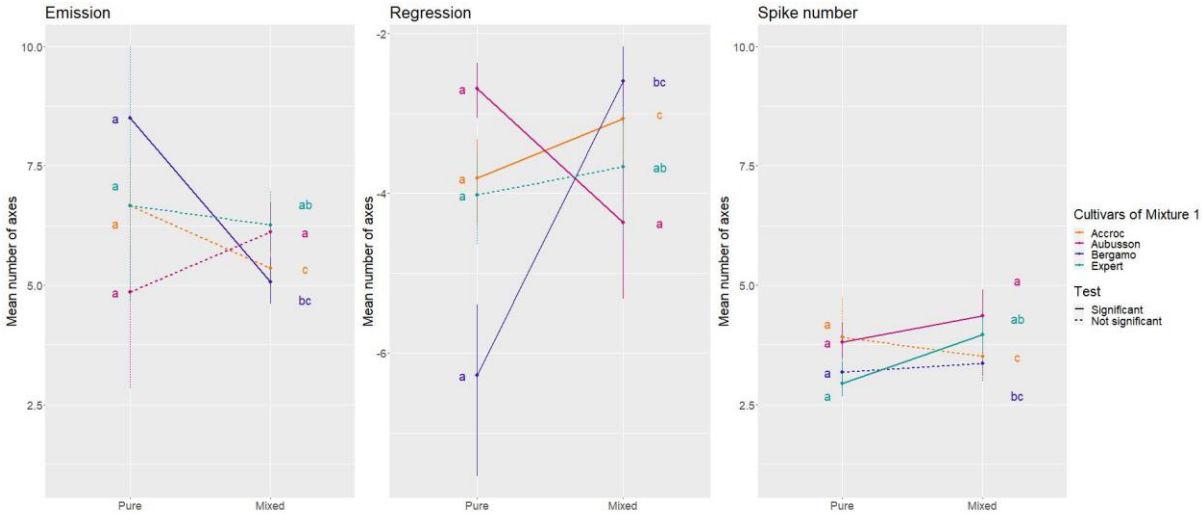

C) Mixture 2 in 2020-2021

Figure S9 - Average tillering dynamics from individual plant data all along the crop cycle for each cultivar of A) Mixture 1 in 2019-2020, B) Mixture 1 in 2020-2021, C) Mixture 2 in 2020-2021 for both pure and mixed stands. Error bars correspond to 95% confidence intervals.

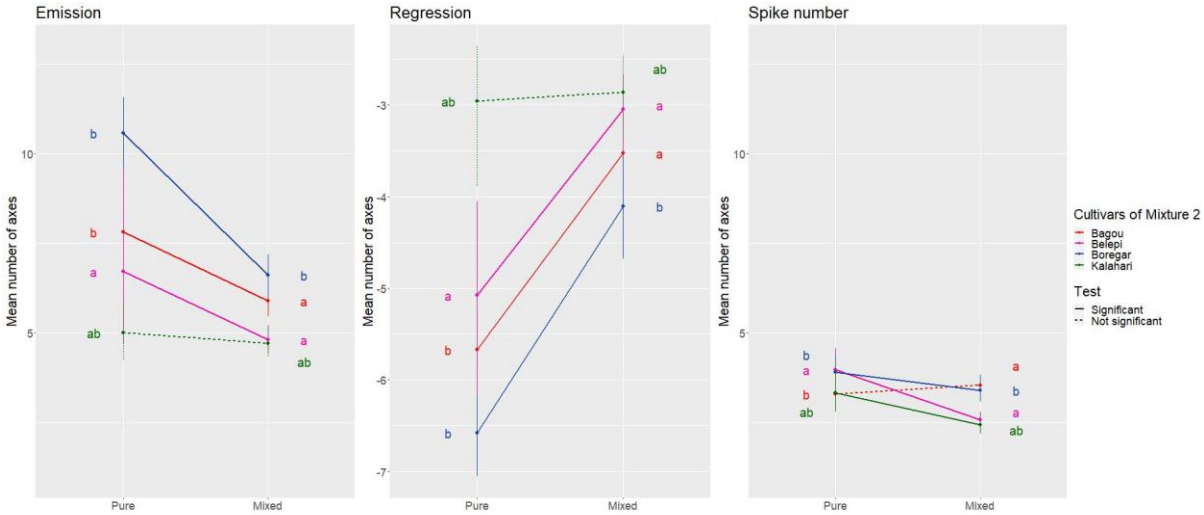
Figure S10




A) Mixture 1 in 2020-2021



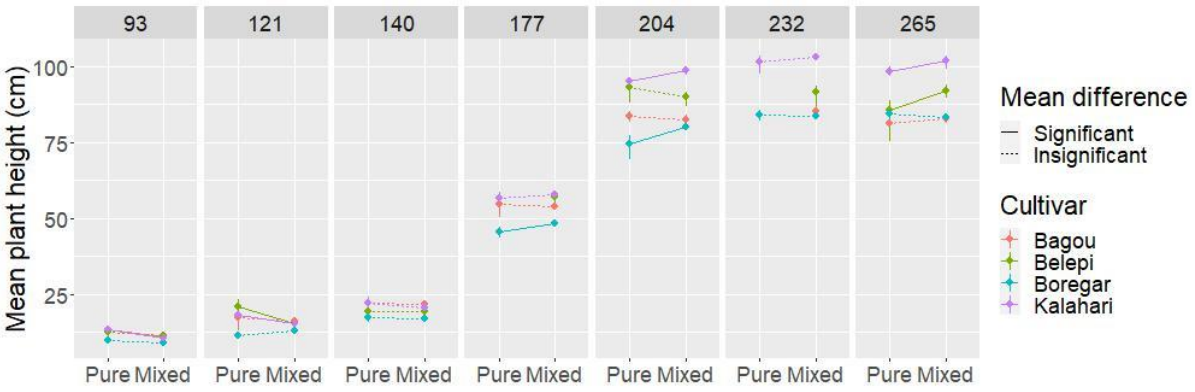

B) Mixture 2 in 2019-2020



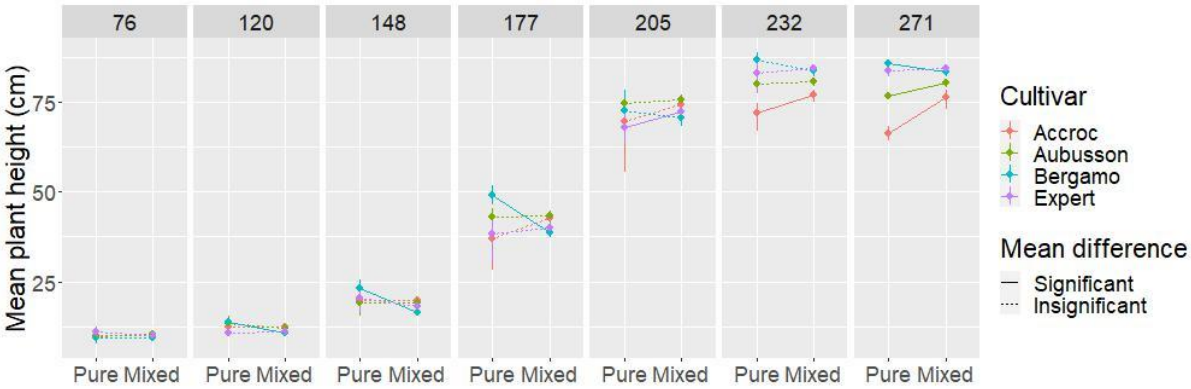

C) Mixture 2 in 2020-2021

Figure S10: Reaction norms of the maximum tiller number (MTN) and the number of regressed tillers (NRT) during the emission and regression phases of axes per plant between pure and mixed stands per cultivar of Mixture 1 in 2019-2020 (A), as well as Mixture 2 in 2019-2020 (B) and 2020-2021 (C). Vertical segments represent confidence intervals at 95%. Letters indicate pairwise differences between cultivars in pure or mixed stands. Line types indicate the statistical significance of the reaction norms.

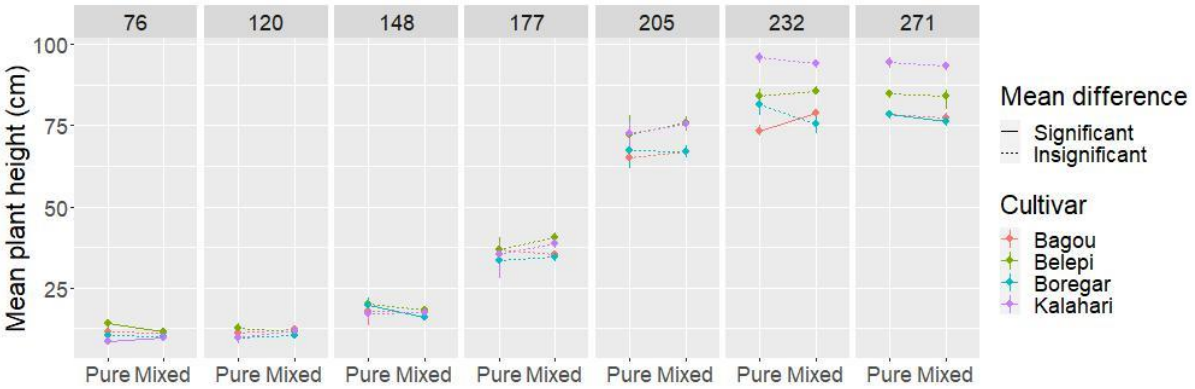
Figure S11




A) Mixture 2 in 2019-2020




B) Mixture 1 in 2020-2021




C) Mixture 2 in 2020-2021

Figure S11: Reaction norms of plant height all along the crop cycle between pure and mixed stands per cultivar for Mixture 2 in 2019-2020 and both mixtures in 2019-2020 and 2020-2021. Line types indicate the statistical significance of the reaction norms.

Figure S12

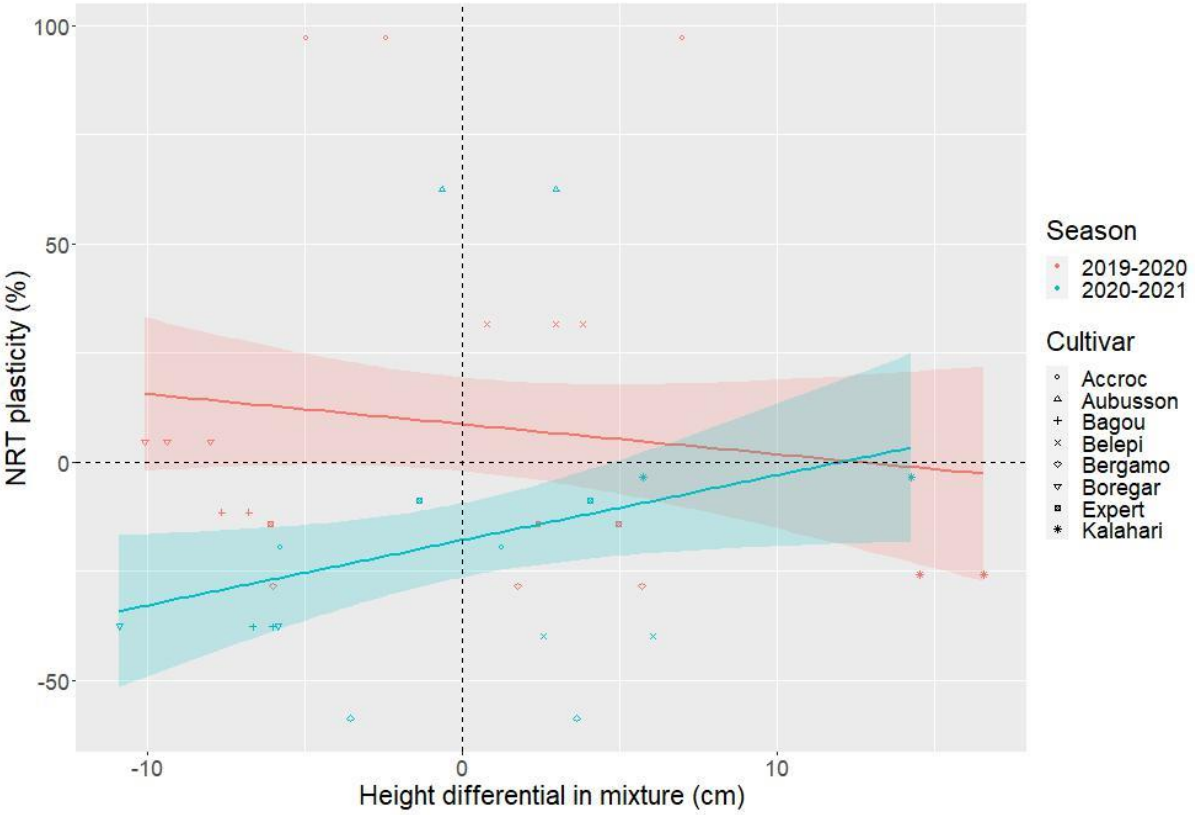



Figure S12: Plasticity of the number of regressed tillers versus height differentials in mixture.