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Multiple abiotic stresses on maize grain yield determination: Additive vs multiplicative effects

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ABSTRACT

Interactions between nitrogen (Ns) and water stress (Ws) effects on annual crops productivity have been widely investigated in Mediterranean-type regions, but not in the humid temperate ones as the central Pampas of Argentina, where early-sown maize crops are usually exposed to severe yield penalties due to the mentioned stresses. Additionally, the recommendation of increasing plant populations promoted by seed companies may push crops to high density stress (HDs), which has usually a multiplicative (i.e. less intensive) and not an additive (i.e. more intensive) effect when combined with other constraints. In current research we re-examined multiple stress effects (HDs, Ns, Ws and the interaction of HDs with either Ns or Ws) on the determinants of grain yield of two maize hybrids, and used relative plant biomass ($SI = 1 - \text{stress}/\text{reference}$) as an integrative seasonal index for describing their intensities. Field experiments included two maize hybrids of contrasting tolerance to stress (high for AX820 and low for AX877), grown under different combinations of stand densities (9 and 12 plants m^{-2}) and N offer (0 and 200 kg $N ha^{-1}$) or water regime (well-watered and water deficit). The SI of combined stresses was always larger than the SI of any individual stress. For the tolerant hybrid, effects of combined abiotic stresses on SI were always multiplicative, whereas for the intolerant one the response intensified (i.e., turned additive or even synergic) under Ws \times HDs. For both hybrids, a single model described the sensitivity of certain traits (number of complete and total florets, number of exposed silks) to the wide range of evaluated SIs, whereas independent models were necessary to accommodate the variation observed in the anthesis-silking interval associated with Ws and Ns. The second pattern was also observed in the case of kernel number per plant and plant grain yield of the intolerant but not of the tolerant hybrid. The former was more sensitive to Ws than the latter. Our results confirm a different sensitivity of the analyzed traits according to the origin of stress and the genotypic variability in these responses.

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1. Introduction

Abiotic stress dominates the list of environmental constraints (e.g. drought, nutrient limitations) that reduce grain yield of annual

crops worldwide (Mueller et al., 2012). These stresses can vary in duration and intensity, and can act simultaneously or sequentially (Loomis and Connor, 1996; Mooney et al., 1991; Sih et al., 1998). Loomis and Connor (1996) suggested that the law of minimum (von Liebig, 1855; de Wit, 1992) is adequate for capturing the effect of multiple stresses in a short time interval (e.g., hours), with growth affected by one stress at a time. In longer periods, however, the final effect of different factors that affect plant growth does not represent the effect of any individual factor but of their interaction. This interaction effect is frequently of multiplicative and not of additive nature when expressed in relative terms respect to potential conditions that maximize grain yield (Sadras, 2005). Moreover, multiplicative effects are usually the result of stresses that take place sequentially along the cycle (Sadras, 2005).

Abbreviations: ASI, anthesis-silking interval; D9, 9 plants m^{-2} ; D12, 12 plants m^{-2} ; Exp, experiment; H, hybrids; HDs, high density stress; KNP, kernel number per plant; N0, no N added; N200, 200 kg $N ha^{-1}$; Ns, N stress; PGY, plant grain yield; SI, stress index; SIa, additive stress index; SIm, multiplicative stress index; SD, stand density; WD, water deficit; Ws, water stress; WW, well-watered; WR, water regime.

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Interactions between N (Ns) and water stress (Ws) have been widely investigated in Mediterranean-type regions where annual crops are commonly exposed to terminal drought (Asseng et al., 2001; Cantero-Martinez et al., 1995; Pala et al., 1996; Palta et al., 1994; Sadras, 2002; van Herwaarden et al., 1998a,b,c). At the crop level, these interactions have often been interpreted within a framework that accounts for the effect of Ns on early growth and Ws on kernel set and grain filling (Fischer, 1979; Passioura, 1977). For these environments, several works have described the effect of Ns \times Ws on grain yield penalties (i.e., the gap between potential and actual yields) of rainfed wheat and barley crops (Sadras, 2004, 2005; Cossani et al., 2010). The magnitude of Ns \times Ws was quantified by using an integrative seasonal index (SI) described as 'co-limitation index', based on the theory of the equally limiting factors proposed by Bloom et al. (1985). This index was calculated as a function of N and water stress indices (e.g., Sadras, 2004) derived from crop simulation models (e.g., CropSyst model; Stöckle et al., 2003) or by the ratio between actual N uptake or water use and the amount required to achieve maximum yields (e.g., Cossani et al., 2010). However, implementation of this methodology could be limited by the several inputs required to run crop simulation models. It can be also limited by the use of a reference maximum N uptake or water use representative of a single potential grain yield, ignoring multisite and inter-annual variations due to factors not related to soil restrictions, as solar radiation and air temperature (Otegui et al., 1996). An alternative way to account for inter-annual variation in potential (unstressed) growth is by expressing actual (stressed) growth relative to the potential growth of each year (Sadras, 2005).

For humid temperate regions as the central Pampas of Argentina, information on the effect of multiple stresses on the productivity of annual crops is scarce. In this region, maize crops are usually exposed to a mid-summer (late December and January) water deficit of variable duration and intensity, which has a predominant negative effect on kernel set of early-sown crops (Hall et al., 1992; Maddonni, 2012). Additionally, maize crops generally do not receive large amounts of N fertilization due to the generally negative effect of this practice on the crop gross margin. This fact, together with intensive land use in the area caused a pronounced decrease in the organic sources of this nutrient as compared to decades ago (Maddonni et al., 1999), when periods under annual crops alternated with periods under regenerative ley pastures. Hence, the occurrence of Ns and/or Ws on maize crops could be expected and magnified by the current high stand densities recommended by seed companies, which may fall within the supra-optimum range (i.e., high density stress; HDs) when crops are exposed to Ns or Ws.

In current research we propose a re-examination of multiple stress effects (HDs combined with either Ns or Ws) on the determination of maize grain yield and its main component, kernel number per plant (KNP), of two modern hybrids a priori classified as contrasting in their grain yield stability across environments (Pagano and Maddonni, 2007; Rossini et al., 2011). For this purpose we included two stand densities within the optimum range recommended for potential growing conditions (Luque et al., 2006). We used relative plant biomass (quotient between stress condition and a reference growing condition) at different growth stages to obtain the SI ($SI = 1 - \text{stress}/\text{reference}$) of individual and combined stresses. We evaluated the response of the determinants of KNP (flore development, the number of exposed silks, and the anthesis-silking interval; Otegui and Andrade, 2000) to a range of SI produced by HDs, Ws, Ns and some of their two-way interactions. This index is also useful to analyze the penalty induced in mentioned traits by a similar intensity of different stresses. We hypothesize that (i) multiple stress factors will have a greater effect on plant biomass than individual stresses, (ii) their com-

bined effect will be similar to the additive or multiplicative effect of individual stresses when stresses occur simultaneously or sequentially in time, respectively, (iii) a similar SI will cause a different response in the determinants of KNP depending on the limiting production factor or the interaction between limiting factors, and (iv) hybrids will differ in the magnitude of the established responses.

2. Materials and methods

2.1. Crop husbandry, treatments and experimental designs

Field experiments were conducted during 2006–2007 (Exp. 1), 2007–2008 (Exp. 2), 2008–2009 (Exp. 3) and 2009–2010 (Exp. 4). Experiments 1 and 2 were performed at the experimental station of the National Institute for Agricultural Technology (INTA) located in Pergamino (33° 56'S, 60° 34'W), Argentina, on a silty clay loam soil (Typic Argiudoll). Treatments included a factorial combination of two single-cross maize hybrids (AX820 and AX877) from Nidera Argentina, two stand densities (9 and 12 plants m^{-2} ; D9 and D12, respectively) and two N levels (N0: control with no added N; N200: a fertilized condition with 200 kg of N ha^{-1} added to the soil). Hybrids were selected based on information provided by Nidera Argentina S.A., which classified them as similar in potential grain yield but of contrasting stability across environments. This information was supported by results obtained in a previous experiment (Rossini et al., 2011), where both hybrids reached a maximum grain yield of ca. 13.2 Mg ha^{-1} , but AX877 was more affected by N \times HDs than AX820. Crops were conducted with supplemental sprinkler irrigation. Experiments 3 and 4 were carried out at the field of the Department of Vegetal Production FA-UBA, Buenos Aires (34° 35' S, 58° 29' W), Argentina, under a polyethylene shelter for avoiding rainfalls, on a deep silty clay loam soil (Vertic Argiudoll). Treatments included a factorial combination of the same single-cross maize hybrids (AX820 and AX877), two stand densities (D9 and D12) and two water regimes (WW: well-watered; WD: water deficit). Crops were conducted with supplemental drip irrigation up to the seven-ligulated leaf stage (V7; Ritchie and Hanway, 1982) in Exp. 3, or V5 in Exp. 4. Subsequent to those stages, WW plots were irrigated frequently (each 5–7 days), with the objective of maintaining plant available soil water content above 50% (Sadras and Milroy, 1996). By contrast, irrigation of WD plots was arrested at mentioned stages, which resulted in a sustained decrease of plant available soil water content along the cycle (Fig. 1) except for some WD plots of AX877 in Exp. 4., that received water from rainfalls at the end of the grain-filling period due to damage on one side of the shelter (Fig. 1d). Other evaluated factors (stand density and hybrid) did not produce changes in soil water contents. All plots were fertilized with 200 kg of N ha^{-1} in Exp. 3 and Exp. 4.

In Exps. 1 and 2, treatments were distributed in a split-plot design with three replicates. N levels were assigned to the main plots, and combinations of stand density (SD) and hybrids (H) to the sub-plots (hereafter termed plots). Plots had six rows, 0.7 m between rows and 18 m length. In Exps. 3 and 4, treatments were distributed in a split-plot design with two replicates. Plots had five rows, 0.5 m between rows and 8 m length. The WR was assigned to the main plots, SD to the sub-plots and H to the sub-subplots (hereafter termed plots).

Sowing was performed manually on 20-Oct 2007 (Exp. 1), 22-Oct 2008 (Exp. 2), 08-Dic 2009 (Exp. 3) and 25-Nov 2010 (Exp. 4), at a rate of 3–4 seeds per hill and thinned to one plant per site at the end of the heterotrophic phase (V3; Pommel, 1990). N was added as urea to all fertilized plots at V6, and incorporated into the soil. All experiments were kept free of weeds by means of chemical con-

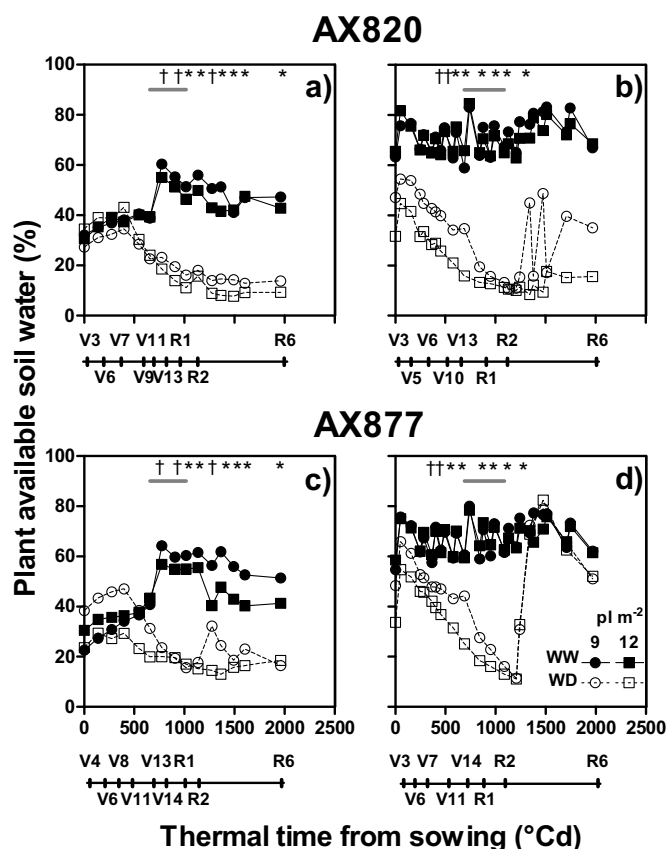


Fig. 1. Available soil water content along the cycle of hybrids AX820 (a and b) and AX877 (c and d) in Exp. 3 (a and c) and Exp. 4 (b and d). Growth stages are indicated on the horizontal solid line at the bottom of each panel. The horizontal line within each panel represents the occurrence of the critical period for kernel set. † and * represent $P < 0.10$ and $P < 0.05$, respectively, for differences in water regimes (WW: well-watered, WD: water deficit).

trols (4 L ha^{-1} of atrazine at 0.5 a.i. + 2 L ha^{-1} of acetochlor at 0.98 a.i.) and hand weeding. Daily maximum and minimum temperatures were registered in the weather station located at 500 m from the experiments (Exps. 1 and 2) or inside the shelter (Exps. 3 and 4), and thermal time was computed from sowing onwards (base temperature of 8°C ; Ritchie and NeSmith, 1991).

2.2. Plant biomass, plant grain yield and kernel number

At V3, 10 (Exp. 1) or 12 (Exps. 2, 3 and 4) consecutive plants of similar size (visual assessment) were tagged at each plot for the non-destructive estimation of mean plant biomass per plot. The date of male (at least one anther visible in the tassel) and female flowering (at least one silk visible in the apical ear; R1) of each tagged plant was recorded. The biomass of these plants was estimated at their corresponding dates of R1 and R2 (ca. $R1 + 15$ days) by means of allometric models broadly used in this species (Andrade et al., 1999, 2002; Vega et al., 2001; Echarte and Tollenaar, 2006; Pagano and Maddonni, 2007; D'Andrea et al., 2008). Details of the non-destructive technique and fitted allometric models were presented in a previous paper for Exp. 1 and Exp. 2 (see Table 1 in Rossini et al., 2011). Models corresponding to Exps. 3 and 4 are presented in Supplemental Table 1.

Tagged plants were individually sampled at physiological maturity (R6) and oven dried to quantify plant grain yield (PGY) and to assess KNP.

2.3. Anthesis-silking interval, silks extrusion and floret development

The interval between anthesis and silking dates (ASI) was computed for each plant tagged for biomass estimation (Section 2.2), and the mean ASI of each plot was obtained as the average of individual ASI values.

The number of silks exposed from the apical ear at $R1 + 4$ days was quantified on 10 (Exp. 1) or 12 (Exps. 2–4) plants per plot, different from those used for biomass estimation (Section 2.2). Silking date (day 1) of these extra plants was registered, and apical ears were collected on day 5. Starting from the base of the ear, three categories of florets were identified along two opposite spikelet rows: (i) florets with silks exposed from the husks, (ii) florets with silks $> 1 \text{ mm}$ (completely developed florets; Otegui and Melón, 1997) but not exposed from the husks, and (iii) florets with silks $< 1 \text{ mm}$ (incomplete florets). The number of completely developed florets per ear was computed in these ears as the product between the number of florets with silks $> 1 \text{ mm}$ (average of two spikelet rows) and the number of spikelet rows per ear (observed at the mid portion of the ear). The number of silks exposed from the apical ear was estimated as the product between the number of exposed silks per spikelet row and the total number of spikelet rows per ear. The number of total florets per ear was estimated as the product between the number of total florets per spikelet row and the total number of rows per ear.

2.4. Integrative seasonal index of abiotic stress effects

Plant biomass at R1 and R2 was used as SI for assessing the intensity of stress caused by evaluated factors on maize growth. For each hybrid in each experiment, mean plant biomass data were ranked within each replicate, and that with the highest value was considered as the reference treatment (i.e., without stress). A SI value of 0 corresponded to the reference treatment, which was always represented by plots of (i) the D9 N200 treatment in Exps. 1 and 2, and (ii) the D9 WW treatment in Exps. 3 and 4. The SI was obtained as described in Eq. (1).

$$SI = 1 - \frac{\text{Plant biomass treatment}_n}{\text{Plant biomass reference treatment}} \quad (1)$$

The SI of main evaluated factors (SD, N, WR level) was calculated and estimated additive (SI_{a_n}) and multiplicative effects (SI_{m_n}) of their combined action were computed as described in Eqs. (2)–(5).

$$SI_{a_1} = SI_{D12} + SI_N \quad (2)$$

$$SI_{a_2} = SI_{D12} + SI_{WD} \quad (3)$$

$$SI_{m_1} = 1 - ((1 - SI_{D12}) \times (1 - SI_N)) \quad (4)$$

$$SI_{m_2} = 1 - ((1 - SI_{D12}) \times (1 - SI_{WD})) \quad (5)$$

In this approach, the SI_{D12} represented the individual effect of HDs and referred to the D12 N200 treatment (Exps. 1 and 2) or the D12 WW treatment (Exps. 3 and 4). The SI_N represented the individual effect of Ns and referred to the D9 N0 treatment (Exps. 1 and 2), whereas the SI_{WD} represented the individual effect of Ws and referred to the D9 WD treatment (Exps. 3 and 4).

2.5. Data analysis

Treatment effects on mean values were evaluated by ANOVA. When necessary (e.g., SI), data were transformed for accomplishing the assumptions of this test. The relationship between variables was evaluated by means of regression analysis, and models fitted

Table 1
Treatments effect on evaluated traits and ANOVA of results. Data correspond to Experiments 1 and 2.

| Experiment | Hybrid | Nitrogenlevel | Stand density (pl m ⁻²) | Biomass per plant (g) | | Florets per ear | | Exposedsilks | ASI ^a (d) | KNP | PGY (g) | | | | |
|--------------------|--------|---------------|-------------------------------------|-----------------------|-------|-----------------|----------|--------------|----------------------|-----|---------|----|----|----|----|
| | | | | R1 | R2 | Total | Complete | | | | | | | | |
| Exp. 1 | AX820 | N200 | 9 | 130.8 | 185.3 | 692 | 687 | 619 | 0.2 | 548 | 157.0 | | | | |
| | | | 12 | 108.1 | 149.9 | 682 | 678 | 612 | 0.6 | 453 | 122.7 | | | | |
| | AX877 | N0 | 9 | 85.9 | 125.2 | 644 | 634 | 551 | 0.9 | 401 | 100.7 | | | | |
| | | | 12 | 77.0 | 109.3 | 649 | 627 | 523 | 1.1 | 355 | 80.9 | | | | |
| | | N200 | 9 | 136.1 | 195.9 | 783 | 777 | 687 | 1.2 | 554 | 161.3 | | | | |
| | | | 12 | 118.2 | 164.0 | 762 | 757 | 662 | 2.2 | 491 | 130.1 | | | | |
| N0 | 9 | 78.8 | 112.4 | 713 | 699 | 615 | 2.3 | 363 | 75.9 | | | | | | |
| | 12 | 65.7 | 97.2 | 701 | 681 | 582 | 2.7 | 296 | 57.7 | | | | | | |
| Exp. 2 | AX820 | N200 | 9 | 95.2 | 149.0 | 704 | 701 | 583 | 0.3 | 453 | 130.2 | | | | |
| | | | 12 | 92.0 | 126.8 | 702 | 701 | 567 | 1.6 | 385 | 98.5 | | | | |
| | | | 9 | 60.5 | 91.8 | 608 | 586 | 468 | 1.5 | 266 | 56.3 | | | | |
| | AX877 | N200 | 12 | 52.4 | 85.2 | 594 | 585 | 441 | 1.7 | 262 | 58.6 | | | | |
| | | | 9 | 170.8 | 198.4 | 766 | 760 | 671 | 1.9 | 510 | 136.2 | | | | |
| | | | 12 | 113.6 | 144.5 | 738 | 729 | 604 | 3.0 | 398 | 99.4 | | | | |
| | | N0 | 9 | 93.1 | 126.1 | 710 | 700 | 537 | 1.8 | 344 | 76.8 | | | | |
| | | | 12 | 79.3 | 108.5 | 693 | 685 | 499 | 3.3 | 260 | 54.6 | | | | |
| | | | Exp. 1 | | | | | | | | | | | | |
| | | | Nitrogen (N) | | | | | | | | | * | ** | ** | ** |
| Hybrid (H) | | | | | | | | | | | ** | ** | | | |
| Stand density (SD) | | | | | | | | | | * | | | * | ** | |
| N × H | | | | | | | | | | | | | | | |
| N × SD | | | | | | | | | | | | | | | |
| H × SD | | | | | | | | | | | | | | | |
| N × H × SD | | | | | | | | | | | | | | | |
| Exp. 2 | | | | | | | | | | | | | | | |
| N | | | | | | | | | * | * | * | * | ** | * | * |
| H | | | | | | | | | ** | ** | ** | ** | ** | * | * |
| SD | | | | | | | | | ** | ** | * | * | * | * | * |
| N × H | | | | | | | | | | | * | * | | | |
| N × SD | | | | | | | | | | | | | | | |
| H × SD | | | | | | | | | * | | | | | | |
| N × H × SD | | | | | | | | | | | | | | | * |

*, **: significant at 0.05 and 0.01, respectively.

^a ASI: anthesis-silking interval, KNP: kernel number per plant, PGY: plant grain yield, N0: control with no added N, N200: fertilized with 200 kg of N ha⁻¹.

using GraphPad Prism 5.0 software (Graphpad Software, San Diego, USA). For the analysis of the response of KNP and its determinants to the SI, trait data were presented as a proportional value to a reference treatment. A value of 1 was always assigned to the D9 N200 or the D9 WW treatments. The SI of plant biomass at R1 or at R2 was used as an independent variable, depending upon the stage of the cycle at which each trait is defined (Otegui and Andrade, 2000). For several traits, the SI built from plant growth during their determining periods would probably represent the magnitude of the stress better than a cumulative value at a given stage, but it brings the restriction of an enhanced number of plant biomass samplings along the cycle and the need of a precise knowledge about the occurrence of each event (e.g., floral initiation) in each genotype (Otegui and Andrade, 2000). Hence, SI of plant biomass at R1 was related to the number of florets and the ASI, whereas SI of plant biomass at R2 was related to the number of exposed silks, KNP, and PGY. Linear functions were fitted to the relationships among traits and SI. Model parameters (i.e., slope and y-intercept) were compared using the extra sum-of-squares F-test ($P < 0.05$) of GraphPad Prism 5.0 (Graphpad Software, San Diego, USA). When necessary, the departure from the 1:1 relationship was evaluated by means of the confidence interval ($P \leq 0.05$) of fitted models.

3. Results

3.1. General effect of treatments on evaluated traits

All main factors representative of abiotic stress had a marked effect on biomass production (Tables 1 and 2). This trait decreased

in response to Ns (between 33 and 41%; across H, Exps. and growth stages), HDs (between 8 and 22%) or Ws (between 17 and 32%). In Exps. 2 and 3, AX877 had larger plant biomass than AX820 (Tables 1 and 2). Interaction effects on biomass production were almost null and were detected only in Exp. 2 (Table 1) for H × SD at R1 ($P < 0.05$).

The effect of treatments varied in intensity across evaluated traits and experiments (Tables 1 and 2). For a developmental trait as the number of florets (both complete and total), the main source of variation was always the H (AX877 > AX820; $0.01 < P < 0.05$). Significant effects ($0.01 < P < 0.05$) of N (Exp. 1 and Exp. 2) were also detected for this trait, as well as of WR ($P < 0.01$) on total florets in Exp. 3. Contrary, there was no effect of SD on these developmental traits. A similar trend was detected for the ASI, with ASI AX877 > ASI AX820. Interaction effects were almost null for these developmental traits (Tables 1 and 2), except for H × N effects ($P < 0.05$) on both total and complete floret numbers in Exp. 2. Ns caused a larger decrease in the number of florets of the AX820 (14–16%) than in those of the AX877 (ca. 7%). Trends were partially different for the number of exposed silks, for which N ($P < 0.01$) and WR ($0.01 < P < 0.05$) modulated silks extrusion, whereas SD effects on this trait were significant ($0.01 < P < 0.05$) only in Exp. 2 and Exp. 3. Hybrid AX877 exhibited a higher ($0.01 < P < 0.05$) number of exposed silks than AX820 in Exps. 1–3. A significant WR × SD effect was registered for the number of exposed silks in Exp. 3 ($P < 0.05$). HDs caused a 22% reduction of this trait under Ws, but only a 6% reduction among well-watered plants.

KNP (main determinant of PGY) decreased 30–35%, 14–39%, and 34–36% in response to Ns, HDs or Ws, respectively (Tables 1 and 2) and consequently, PGY was also affected by Ns (45–47%), HDs

Table 2
Treatments effect on evaluated traits and ANOVA of results. Data correspond to Experiments 3 and 4.

| Experiment | Hybrid | Waterregime | Stand density (pl m ⁻²) | Biomass per plant (g) | | Florets per ear | | Exposed silks | ASI ^a (d) | KNP | PGY (g) |
|--------------------|--------|-------------|-------------------------------------|-----------------------|-------|-----------------|----------|---------------|----------------------|--------------------|---------|
| | | | | R1 | R2 | Total | Complete | | | | |
| Exp. 3 | AX820 | WW | 9 | 125.5 | 170.0 | 671 | 661 | 601 | 1.7 | 455 | 128.5 |
| | | | 12 | 117.9 | 143.7 | 694 | 674 | 534 | 1.1 | 328 | 84.4 |
| | WD | 9 | 102.9 | 134.1 | 662 | 639 | 557 | 2.5 | 279 | 58.9 | |
| | | 12 | 99.3 | 117.1 | 686 | 644 | 451 | 3.4 | 224 | 58.9 | |
| | AX877 | WW | 9 | 141.9 | 179.1 | 838 | 832 | 776 | 2.5 | 527 | 125.5 |
| | | | 12 | 133.3 | 154.4 | 812 | 809 | 757 | 3.1 | 317 | 75.3 |
| WD | 9 | 117.5 | 163.3 | 802 | 777 | 694 | 4.0 | 412 | 93.7 | | |
| | 12 | 99.1 | 119.7 | 720 | 706 | 529 | 5.9 | 153 | 31.1 | | |
| Exp. 4 | AX820 | WW | 9 | 117.2 | 153.4 | 711 | 701 | 636 | 0.0 | 358 | 91.8 |
| | | | 12 | 97.0 | 120.6 | 665 | 652 | 583 | 1.6 | 228 | 58.4 |
| | WD | 9 | 82.4 | 101.2 | 695 | 675 | 564 | 2.0 | 195 | 53.0 | |
| | | 12 | 72.1 | 92.6 | 684 | 660 | 525 | 2.7 | 179 | 45.9 | |
| | AX877 | WW | 9 | 130.6 | 154.8 | 858 | 848 | 746 | 0.4 | (163) ^b | (39.8) |
| | | | 12 | 127.7 | 140.1 | 844 | 839 | 764 | 0.9 | (13) | (4.1) |
| WD | 9 | 92.5 | 131.6 | 845 | 839 | 769 | 1.7 | (236) | (58.6) | | |
| 12 | 76.3 | 97.3 | 819 | 792 | 508 | 3.4 | (51) | (13.2) | | | |
| Exp. 3 | | | | | | | | | | | |
| Water regime (WR) | | | | * | ** | ** | | ** | | * | * |
| Stand density (SD) | | | | * | * | | | ** | | * | * |
| Hybrid (H) | | | | * | * | * | ** | | ** | | |
| WR × SD | | | | | | | ** | | | | |
| WR × H | | | | | | | * | | | | |
| SD × H | | | | | | | | | | | |
| WR × SD × H | | | | | | | | | | | |
| Exp. 4 | | | | | | | | | | | |
| WR | | | | * | | | | * | | | |
| SD | | | | | | | | | | | |
| Hybrid (H) | | | | | | ** | ** | | | - | - |
| WR × SD | | | | | | | | | | - | - |
| WR × H | | | | | | | | | | - | - |
| SD × H | | | | | | | | | | - | - |
| WR × SD × H | | | | | | | | | | - | - |

* and **: significant at 0.05 and 0.01, respectively.

^a ASI: anthesis-silking interval, KNP: kernel number per plant, PGY: plant grain yield, WW: well-watered, WD: water deficit.

^b Data in parenthesis were not included in the ANOVA due to heat stress effects on pollen viability and kernel set of hybrid AX877.

(21–38%) or Ws (34–41%). In Exp. 4, data of AX877 were excluded from the analysis, because a brief period of heat stress affected pollen viability and kernel set of this hybrid with delayed anthesis (Rossini et al., 2012). No interaction effect was detected for this trait.

PGY had a significant correlation ($P < 0.05$) with almost all evaluated traits, but r values were consistent across experiments only for its association with (i) KNP ($r \geq 0.97$), (ii) plant biomass at R2 ($r \geq 0.89$) and R1 ($r \geq 0.78$), and (iii) the number of exposed silks ($r \geq 0.62$). The response of PGY to the total number of florets ($r = 0.61$ for Exps. 1 and 2; $r = 0.41$ for Exps. 3 and 4), the number of complete florets ($r = 0.65$ for Exps. 1 and 2; $r = 0.45$ for Exps. 3 and 4), and the ASI ($r = -0.55$ for Exps. 1 and 2; $r = -0.42$ for Exps. 3 and 4) was highly significant for Exps. 1 and 2, but not for Exps. 3 and 4.

3.2. Integrative seasonal index of stress

All evaluated factors (SD, N and WR) produced a significant ($P < 0.05$) effect on SI (Table 3), except SD in Exp. 4. A consistent N × SD interaction effect was detected for the SI at R2 ($P < 0.05$ in both experiments), which was not the case for other interactions. This interaction detected that HDs caused a larger increase in SI for the N200 than for the N0 treatment. In contrast, the significant ($P < 0.05$) WR × SD interaction registered in Exp. 3 represents an enhanced negative effect of HDs under WD than under WW conditions.

For the experiments included in current research (Table 3), average SI rose to (i) ca. 0.12 at R1 and 0.17 at R2 in response to the individual effect of HDs (average across all D12 N200 and D12 WW conditions), (ii) 0.39 at R1 and 0.37 at R2 in response to the individual effect of Ns (average across all D9 N0 conditions), and (iii) 0.24 at R1 and 0.20 at R2 in response to the individual effect of Ws (average across all D9 WD conditions). The SI of combined stresses was predominantly driven by the individual SI of Ns or Ws rather than by the individual SI of HDs (Table 3).

The effects of combined stresses (Ns × HDs and Ws × HDs) on SI were always larger than any individual stress, but usually not larger than the estimated SIa of individual stresses, with the exception of Ws × HDs (D12 WD) treatments of AX877 (Table 3). For each hybrid, a significant linear model ($P < 0.05$) was fitted to the relationship between actual interaction effects on SI and SI_m at R1 and R2 (Fig. 2). Most data of AX820 were close to the 1:1 relationship, indicative of a predominantly multiplicative effect of stresses. The theoretical model of estimated additive effects did not depart markedly from the 1:1 relationship for SIs lower than 0.40, and most SIs of AX820 were below or close to this threshold. Contrarily, actual SIs of AX 877 were (i) higher than 0.40 and fell close to the 1:1 relationship for Ns × HDs, and (ii) lower than 0.40 and fell above the 1:1 relationship for Ws × HDs. The first pattern was indicative of predominant multiplicative effects, whereas the second pattern represented a synergy between Ws and HDs.

Table 3
Stress index (SI) of individual factors and their interactions at two growth stages (R1 and R2). The estimated additive effects (Sla) and estimated multiplicative effects (Slm) of individual factors are indicated.

| Experiment | Hybrid | Nitrogen level | Stand density (pl m ⁻²) | SI R1 | Sla ₁ R1 | Slm ₁ R1 | SI R2 | Sla ₁ R2 | Slm ₁ R2 | Experiment | Hybrid | Water regime | Stand Density (pl m ⁻²) | SI R1 | Sla ₂ R1 | Slm ₂ R1 | SI R2 | Sla ₂ R2 | Slm ₂ R2 | | |
|--------------------|--------|-------------------|--|----------|------------------------|------------------------|----------|------------------------|------------------------|--------------------|--------|--------------|--|----------|------------------------|------------------------|----------|------------------------|------------------------|------|------|
| Exp. 1 | AX820 | N200 ^a | 9 | 0.00 | | | 0.00 | | | Exp. 3 | AX820 | WW | 9 | 0.00 | | | 0.00 | | | | |
| | | | 12 | 0.17 | | | 0.19 | | 0.15 | | | | | | | | | | | | |
| | | N0 | 9 | 0.34 | | | 0.32 | | 0.21 | | | | | | | | | | | | |
| | | | | 12 | 0.41 | 0.51 | 0.45 | 0.41 | 0.51 | | 0.45 | | WD | 9 | 0.18 | | | 0.21 | | | |
| | AX877 | N200 | 9 | 0.00 | | | 0.00 | | | | Exp. 4 | AX877 | | WW | 9 | 0.00 | 0.25 | 0.23 | 0.31 | 0.36 | 0.33 |
| | | | 12 | 0.13 | | | 0.16 | | 0.14 | | | | | | | | | | | | |
| N0 | | 9 | 0.42 | | | 0.43 | | 0.09 | | | | | | | | | | | | | |
| | | | 12 | 0.52 | 0.55 | 0.50 | 0.50 | 0.59 | 0.52 | | | WD | 9 | 0.17 | | | 0.33 | 0.23 | 0.22 | | |
| AX820 | N200 | 9 | 0.00 | | | 0.00 | | | Exp. 4 | AX820 | | | WW | 9 | 0.00 | | | 0.00 | | | |
| | | 12 | 0.03 | | | 0.15 | | 0.21 | | | | | | | | | | | | | |
| | N0 | 9 | 0.36 | | | 0.38 | | 0.34 | | | | | | | | | | | | | |
| | | | 12 | 0.45 | 0.39 | 0.38 | 0.43 | 0.53 | | 0.47 | | WD | 9 | 0.30 | 0.47 | 0.42 | 0.40 | 0.55 | 0.48 | | |
| AX877 | N200 | 9 | 0.00 | | | 0.00 | | | | Exp. 4 | AX877 | | WW | 9 | 0.00 | | | 0.00 | | | |
| | | 12 | 0.33 | | | 0.27 | | 0.09 | | | | | | | | | | | | | |
| | N0 | 9 | 0.45 | | | 0.36 | | 0.15 | | | | | | | | | | | | | |
| | | | 12 | 0.54 | 0.78 | 0.63 | 0.45 | 0.63 | 0.53 | | | WD | 9 | 0.29 | 0.31 | 0.30 | 0.37 | 0.24 | 0.23 | | |
| | | | | | | | | | | | | | | 12 | 0.42 | 0.31 | 0.30 | 0.37 | 0.24 | 0.23 | |
| Exp. 1 | | | | | | | | | | | Exp. 3 | | | | | | | | | | |
| Nitrogen (N) | | | | * | | | * | | | Water regime (WR) | | | | * | | | * | | | | |
| Hybrid (H) | | | | | | | | | | Stand density (SD) | | | | ** | | | * | | | | |
| Stand density (SD) | | | | * | | | ** | | | Hybrid (H) | | | | * | | | | | | | |
| N × H | | | | | | | | | | WR × SD | | | | | | | | | | | |
| N × SD | | | | | | | * | | | WR × H | | | | | | | | | | | |
| H × SD | | | | | | | | | | SD × H | | | | | | | | | | | |
| N × H × SD | | | | | | | | | | WR × SD × H | | | | | | | | | | | |
| Exp. 2 | | | | | | | | | | Exp. 4 | | | | | | | | | | | |
| Nitrogen (N) | | | | * | | | * | | | Water regime (WR) | | | | * | | | | | | | |
| Hybrid (H) | | | | | | | | | | Stand density (SD) | | | | | | | | | | | |
| Stand density (SD) | | | | ** | | | *** | | | Hybrid (H) | | | | | | | | | | | |
| N × H | | | | | | | | | | WR × SD | | | | | | | | | | | |
| N × SD | | | | | | | ** | | | WR × H | | | | | | | | | | | |
| H × SD | | | | | | | | | | SD × H | | | | | | | | | | | |
| N × H × SD | | | | | | | | | | WR × SD × H | | | | | | | | | | | |

*, **, ***: significant at 0.05, 0.01 and 0.001, respectively.

^a N0: control with no added N, N200: fertilized with 200 kg of N ha⁻¹, WW: well-watered, WD: water deficit.

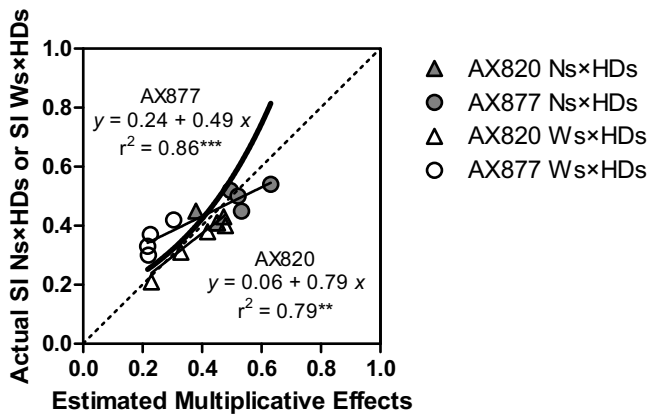


Fig. 2. Response of the actual stress index (SI) of combined stresses (interaction) to the estimated multiplicative effect of evaluated stresses. Ns: nitrogen stress; Ws: water stress; HDs: high density stress. Grey and empty symbols are for Ns × HDs and for Ws × HDs, respectively. Thin solid lines represent models fitted to the data set of AX820 and AX877. Thick line describes the corresponding estimated additive effect for each estimated multiplicative effect. Dotted line represents the 1:1 relationship between actual effects and estimated multiplicative effects.

3.3. Grain yield determination and the integrative seasonal index of stress

Almost all traits (in relative scale) had a significant response ($P < 0.05$) to SI (Figs. 3 and 4). The only exception corresponded to ASI data of AX820 in Exps. 1 and 2 (Fig. 3e). For traits as the total number of florets (Fig. 3a and b), the number of complete florets (Fig. 3c and d), and the number of exposed silks (Fig. 4a and b), a single model accommodated all the variation produced by N, WR and SD. The same trend was registered for PGY of the AX820 (Fig. 4e), but not for PGY of the AX877 (Fig. 4f). For the latter, as well as for the ASI (Fig. 3e and f) and KNP (Fig. 4c and d) of both hybrids, two significantly different ($P < 0.05$) models were established, one for Exps. 1 and 2, and the other for Exps. 3 and 4.

In spite of the high significance level of most correlations, the large variation registered in the SIs had an almost null effect on the total number of florets, evident in all slope values ≤ 0.21 (absolute terms). The effect increased for the number of exposed silks (slope = 0.39), and reached values of slope ≥ 0.86 for all other traits. When independent models were fitted between groups of experiments, slopes of those computed for Exps. 3 and 4 were always larger than slopes of those computed for Exps. 1 and 2. In other words, a similar SI produced a larger effect on ASI, KNP and PGY when it was associated to Ws than when it was associated to Ns. Additionally, the effects of SI on these traits were larger for the AX877 hybrid than for the AX820 hybrid for (i) KNP, in Ns × HDs as well as in Ws × HDs ($0.05 < P < 0.10$), and (ii) PGY, in Ws × HDs ($P < 0.05$). By contrast, the effect of SI on ASI was larger for AX820 than for AX77, but only for Ws × HDs ($P < 0.05$).

4. Discussion

In this paper we have re-examined multiple stress effects on the determinants of KNP and PGY in maize crops. We have used the SI for HDs, Ns, Ws and some of their interactions to assess the response of floret development, the number of exposed silks, the ASI and KNP to individual and combined stresses. Two hybrids a priori classified as contrasting in their grain yield stability across environments (Pagano and Maddonni, 2007; Rossini et al., 2011) were analyzed. We hypothesized that multiple abiotic stresses will have a greater impact on plant biomass than individual stresses. We confirmed that SIs of combined stresses were always larger than those of individual stresses (i.e., the ‘law of minimum’ was never

verified), which supported the proposed hypothesis. In the second hypothesis, we stated that the combined effect of stresses will be similar to the additive or multiplicative effects of individual stresses when stresses occur simultaneously or sequentially in time; respectively. For AX820, SIs of Ns × HDs and Ws × HDs were adequately described as multiplicative effects (Fig. 2). However, for the range of SIs estimated for this genotype, additive effects could also describe these two way interactions adequately, because the differences in SIs between both approaches (i.e., additive and multiplicative) tend to be very small at low SI values. For AX877, the SI of Ns × HDs followed a similar trend and was well represented by the ‘multiplicative effects’ pattern; but the SI of Ws × HDs revealed a synergic effect between these constraints (i.e., a penalty larger than the additive effect). The multiplicative effect detected for all Ns × HDs conditions was the result of a sequential effect of these stresses on plant biomass production, because the occurrence of Ns (V8) preceded that of HDs (V12), as described by Rossini et al. (2011). Contrarily, the combined effect of HDs and Ws (i.e., Ws × HDs) was the result of their simultaneous incidence on plant biomass production from V9 onwards (data not shown), which caused an additive or even synergic effect of both factors on this trait for the hybrid a priori classified (Rossini et al., 2011) as intolerant to N × stand density stress (AX877). Collectively, our results do not allow rejecting the second hypothesis, but added valuable information at the crop level of the relationship between the temporal occurrence of abiotic stresses and its impact on multiple stress effects (i.e., additive or multiplicative effects). Multiplicative effects of Ns and Ws on crop productivity are the expected result for winter grain crops cultivated in Mediterranean-type regions (Fischer, 1979; Passioura, 1977). However, for rainfed maize crops cultivated in humid or semi-humid regions with low amounts of N fertilizer, either additive or multiplicative effects could be expected. The predominance of one type of response or the other (i.e. additive effects or multiplicative effects) may recognize different origins, as inter-annual oscillations of spring rainfalls along decadal periods (Penalba and Vargas, 2004), as well as extreme phenomena as those produced by ‘El Niño Southern Oscillation’ (Messina et al., 1999). The former is more representative of semi-humid regions and the latter of the humid ones.

In the third hypothesis we proposed that a similar decrease in relative plant growth will cause a different response in evaluated traits depending on the limiting production factor or the interaction between limiting factors. On one hand, a single model gave an adequate fit to the response of several developmental traits (total number of florets, completely developed florets, and number of exposed silks) to SI based on plant biomass close to R1, independently of the type of stress experienced by plants. On the other hand, independent models were necessary for explaining the variation caused by WR × SD effects with respect to the variation caused by N × SD effects on traits as the ASI, KNP and PGY. The described responses did not allow for a conclusive statement on the proposed hypothesis, but they deserve several considerations. Firstly, early studies on maize kernel number determination (Fischer and Palmer, 1984) referred to the relative variation in KNP caused by a stress of similar duration and intensity applied along the cycle, but to our knowledge there was no study reporting on the relative response of all developmental and production traits to a wide variation in stress intensities caused by individual or combined abiotic factors, and synthesized in an index (i.e., SI). As expected (Fischer and Palmer, 1984), stress sensitivity (i.e., slope of the relationships in Figs. 3 and 4) increased as the cycle progressed up to R2. In agreement with previous reports (Edmeades et al., 1993; Otegui, 1997), sensitivity to abiotic stress of early-established developmental traits as total floret number and the number of completely developed florets was markedly smaller (slope ≤ 0.21) than that registered for the associated biomass production. Conse-

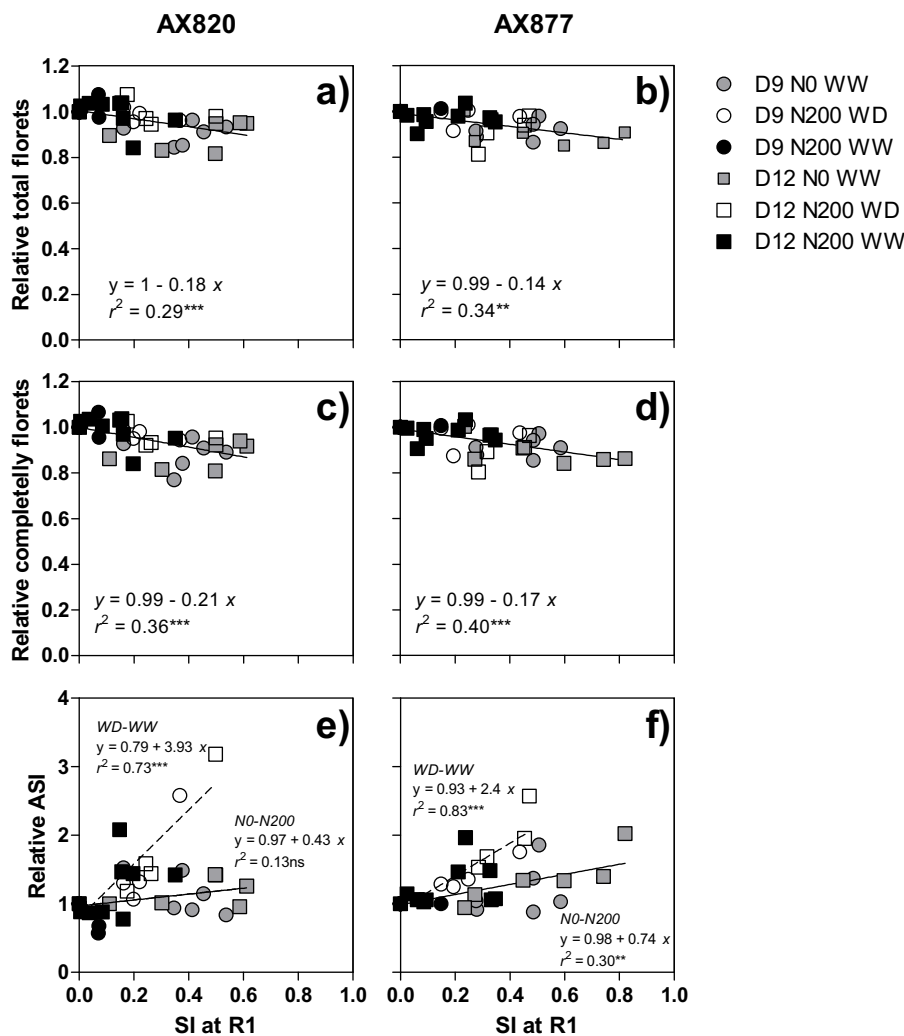


Fig. 3. Relative number of total florets (a and b), completely developed florets (c and d) and anthesis-silking interval (ASI) (e and f) of hybrids AX820 (a, c and e) and AX877 (b, d and f) as a function of stress index (SI) at R1 stage. D9: 9 pl m⁻²; D12: 12 pl m⁻²; N200: 200 kg N ha⁻¹; N0: no N added; WW: well-watered; WD: water deficit. Each data correspond to a replicate (plot).

quently mentioned developmental traits were less dependent on plant biomass than late-established reproductive traits as ASI, KNP and PGY. Additionally, for this second group of traits a similar SI usually produced a larger effect when it was associated with Ws than when it was associated with Ns. Such a response does not support the concept of a single model for accommodating all abiotic stress effects on KNP mediated by plant growth (Andrade et al., 2002). Contrary, it reinforces the idea of additional effects of stress on biomass partitioning to the ear, which make necessary the fit of independent models (e.g., one for control plots and another one for Ns plots) to the response of KNP to plant growth rate during the critical period around flowering of some genotypes (D'Andrea et al., 2006, 2008).

Finally, we stated that the hybrids will differ in the magnitude of the established responses. This hypothesis was tested by analyzing the sensitivity (i.e., the slope of the fitted models) of different traits to variations in the SI (Figs. 3 and 4). For certain traits (number of complete and total florets, number of exposed silks) both hybrids behaved similarly in response to the wide range of evaluated SIs (i.e., their sensitivity was similar). However, there were differential responses between hybrids for traits such as ASI, KNP and PGY. An independent model was necessary for fitting the variation produced by Ws in ASI of both hybrids, which exhibited a contrasting sensitivity (AX820 > AX877). This particular effect held for KNP, but

the sensitivity level (AX877 > AX820) was reversed. The enhanced sensitivity of production traits (KNP and PGY) to Ws exhibited by the most intolerant hybrid (AX877) may recognize different causes; for instance, a more pronounced decline in biomass partitioning to the ear (Echarte and Tollenaar, 2006) or a sharper decline in enzyme activity responsible of sugar transport into kernels (Zinselmeier et al., 1995). Independently of the underlying cause, our results confirmed (i) the contrasting sensitivity of reproductive and production traits to different abiotic stresses (Edmeades et al., 1993; Munaro et al., 2011), even when the effect of these stresses was expressed by means of a growth-based stress index, and (ii) the need of independent models for fitting the variation in these traits introduced by genotypes (D'Andrea et al., 2006, 2008).

5. Conclusions

The use of a stress index (SI) based on relative plant biomass allowed the comparison of individual and combined abiotic stresses on maize reproductive and production traits. We tested individual and multiple stress effects obtained by combinations of HDs with Ns or Ws, and demonstrated that interaction effects were always larger than individual effects. Moreover, the predominant response was of the multiplicative type. This response, however, depended upon the intensity of stress experienced by plants, but also (and chiefly)

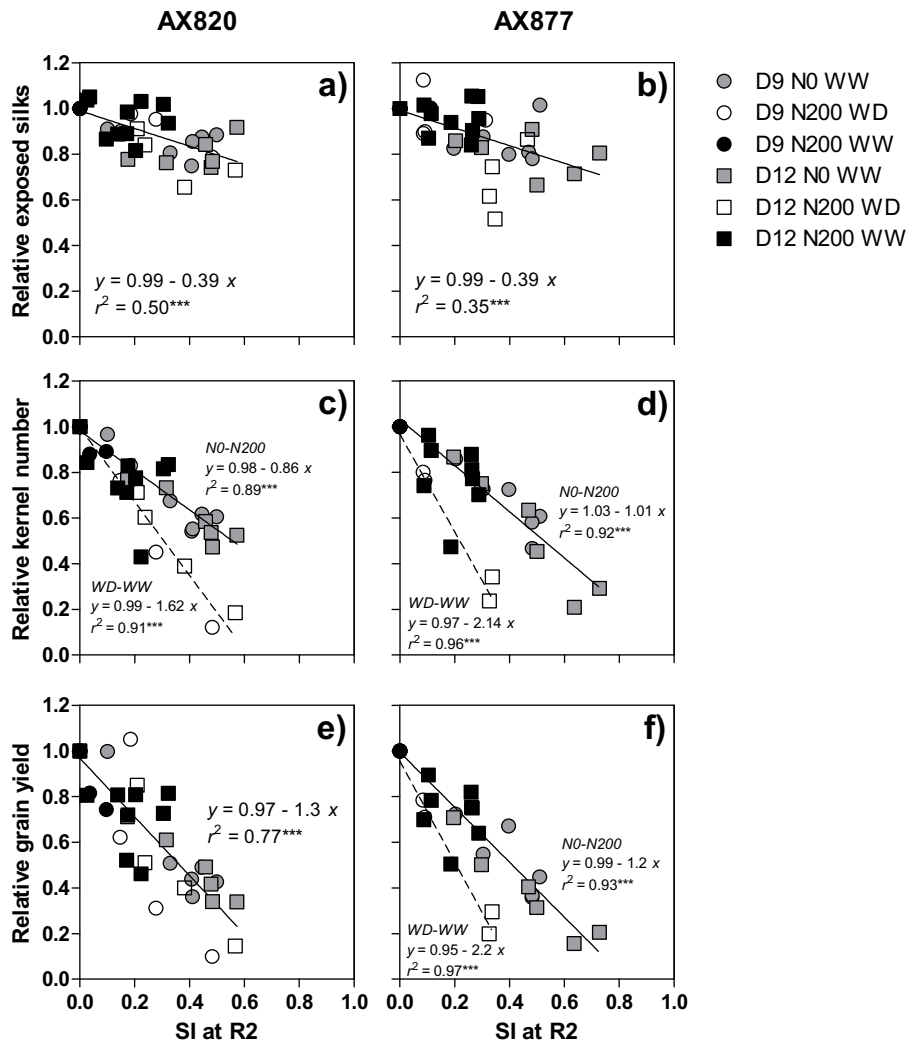


Fig. 4. Relative number of the exposed silks (a and b), kernel number per plant (c and d) and grain yield per plant (e and f) of hybrids AX820 (a, c and e) and AX877 (b, d and f) as a function of stress index (SI) at R2 stage. D9: 9 pl m⁻²; D12: 12 pl m⁻²; N200: 200 kg N ha⁻¹; N0: no N added; WW: well-watered; WD: water deficit. Each data correspond to a replicate (plot). Data of AX877 in Exp. 4 (d and f) were excluded from the analysis.

upon the evaluated genotype. Interestingly, we detected multiplicative or additive effects at relatively low levels of stress, but only multiplicative effects at high levels. For the latter, the intensity of stress was predominantly driven by Ns, which apparently anticipated the effects of crowding produced by enhanced stand density (sequential action). In other words, our results suggest that the impact of multiple abiotic stresses on crop growth seems to depend strongly on the opportunity of stress influence on the crop. Genotypic differences add uncertainty to the responses. For instance, for the most tolerant hybrid (AX820) effects of combined abiotic stresses (Ns × HDs or Ws × HDs) were always multiplicative, whereas for the less tolerant hybrid (AX877) the response intensified (i.e., turned additive or even synergic) under Ws × HDs. This difference calls attention on the importance of the target environment for breeding purposes, particularly the identification of the predominant stress (Ns or Ws) that may accompany HDs for the correct selection of ‘stress tolerant’ hybrids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fcr.2016.07.004>.

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