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DROUGHT STRESS

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# Does the plant growth regulator paclobutrazol enhance root growth of maize exposed to drought stress during flowering?

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## Abstract

Due to climate change, crop production will increasingly be affected by water limitation, causing remarkable decreases in grain yields of cereals. Plant growth regulators such as paclobutrazol (PAC) have been shown to protect plants from detrimental impacts of drought stress, and improvement of root growth and antioxidant activity were identified as main reasons for their positive effect. A container experiment was conducted with two maize (*Zea mays* L.) cultivars, Galactus and Fabregas, to investigate how PAC application affects root growth and grain yield under stress conditions. At growth stage V8, the plants were treated once with PAC (0, 2, or 3 mg PAC per plant), and concomitantly reduction in soil water content commenced until 30–35% of the maximum water-holding capacity (WHC) was achieved. The plants were exposed to this drought condition for three weeks during flowering as the critical period for kernel setting. Both factors, PAC application and drought stress, caused decreases in plant height, whereas total leaf area was unchanged and transpiration rate was significantly reduced by water limitation only. Flowering was almost unaffected by PAC treatment; yet, drought stress significantly delayed start of silking. The straw yield was decreased due to PAC and drought stress, and an improvement of the harvest index was obtained for drought-stressed Galactus plants with PAC application. Grain yield was unaffected by PAC application, whereas drought stress caused significant decreases by 15% on average of both cultivars. The kernel number of drought-stressed Galactus plants was increased after PAC treatment, but concurrently smaller kernels were produced. Water limitation generally decreased kernel number. Drought-stressed Fabregas plants consumed less water after PAC treatment, resulting in significant improvements of water-use efficiency ($WUE_{grain}$) during silking and thus most likely alleviating stress intensity. For both cultivars, PAC treatment and water limitation showed almost no significant impact on root dry matter, root length density, and root surface area, either determined for different soil layers down to 80 cm or on a per-plant basis. It is concluded that grain yield performance of maize plants, exposed to water limitation during flowering, was not source-limited but sink-limited. Consequently, even if PAC can cause improvement of antioxidant activity and photosynthesis, due to sufficient availability of assimilates in the maize kernels a positive effect on grain yield is improbable. Considering source–sink relationships during flowering and kernel set, enhanced root growth due to PAC treatment did apparently not occur.

**KEYWORDS**
grain yield, kernel setting, root morphology, source–sink relations, water-use efficiency, *Zea mays*

### Key Points

- Plant growth regulators such as paclobutrazol (PAC) have been shown to protect plants from detrimental impacts of drought stress; yet, studies were often conducted during vegetative growth only and grain yield data at maturity are hardly available.
- Improvement of root growth was identified as one reason for their positive effects.
- Two maize cultivars, subjected to drought stress during flowering, showed no effect of PAC application on root parameters such as dry matter, length density, and surface area, and also grain yield was unaffected.
- The missing positive impact of PAC can be explained in terms of the prevailing source–sink relations during flowering and kernel setting.

## 1 | INTRODUCTION

The plant growth regulator paclobutrazol (PAC) belongs to the triazole family (Desta & Amare, 2021) with most pronounced effects on shoot growth and plant height. It inhibits the biosynthesis of gibberellins, resulting in reduced elongation growth of the internodes and finally in a decrease in plant height (e.g. Hütsch & Schubert, 2018, 2021a, 2021b, 2022a; Iremiren et al., 1997; Kamran, Cui, et al., 2018; Schluttenhofer et al., 2011). Effects on root growth may be either inhibitory or stimulatory, depending on the plant species tested and the concentration of the triazole compound used (Fletcher et al., 2000). A stimulation in root growth may be related to the increased partitioning of assimilates towards the roots due to a decreased demand in the shoots (Fletcher et al., 2000; Kamran, Wennan, et al., 2018). Although the effects on root growth vary, a higher root-to-shoot ratio is usually a characteristic of triazole-treated plants, primarily due to the reduction in shoot growth (Davis et al., 1988; Fletcher et al., 2000; Kamran, Wennan, et al., 2018).

Root traits such as biomass, length, density, surface area, and depth have been shown to contribute towards water-stress avoidance (Comas et al., 2013; Hund et al., 2009; Klein et al., 2020; Zhan et al., 2015). Particularly under water-limited conditions, an improved root system with increased length and surface area enables the exploration of a larger soil volume for water acquisition. In addition to less water demand of smaller plant shoots, an increased water uptake ability can relieve water limitation. In previous studies that examined triazole application to crops under drought stress, the focus was on the effects on shoots, roots were hardly investigated as their preparation is very laborious if soil culture is employed. Under water limitation, imposed throughout the entire growth period, PAC-treated maize plants showed a significant 20% increase in grain yield and single kernel weight, but no effect on number of kernels per cob (Bayat & Sepehri, 2012). In this study, roots were not investigated. If root studies were conducted, often small plants in the seedling stage were investigated, or in some cases water stress was induced by application of PEG in hydroponics. In maize seedlings, PAC altered root anatomy by causing an increase in root diameter, whereas root length was unaffected (Barnes et al., 1989). After application of uniconazole to maize plants in hydroponics, under drought stress an increase of root biomass, length, surface area, diameter, and volume was observed in an early vegetative growth stage V5 (Wang et al., 2022). During the seedling stage of soybean, comparable results were obtained after uniconazole application (Yan et al., 2013). On the contrary, tomato plants with deficit-irrigation showed decreases in root area, root dry weight, and root-to-shoot ratio after PAC application (Pal et al., 2016). In studies of Urfan et al. (2022), two PAC-treated maize cultivars were exposed either to early or late drought stress, which was 15 to 35 DAS (days after sowing) or starting with 54 DAS, respectively. Depending on the maize cultivar tested, root parameters were unaffected or partly improved when PAC application was combined with stress. Unfortunately, grain yield data are missing for physiological maturity of maize plants (Urfan et al., 2022). In a semiarid climate under field conditions with periodic drought events, Kamran, Wennan, et al. (2018) investigated the effect of PAC on maize root morphology and grain yield. PAC retarded shoot growth, promoted root growth and development, and resulted in increased grain yields due to enhanced grain filling. It was concluded that higher grain yields relied on the improved rooting system, which enabled better water uptake resulting in less drought stress intensity and finally in less detrimental effects of drought on grain yield (Kamran, Wennan, et al., 2018). However, in this study no information is available on the growth stages when drought stress prevailed nor on stress intensity, and the PAC effects on maize plants under well-watered conditions were not tested in these field studies.

Apart from changes in plant morphology, the most pronounced physiological effect of triazoles has been observed with the enzymes related to antioxidant activity, thus protection of the photosynthetic machinery from damage due to reactive oxygen species (Bayat & Sepehri, 2012; Chandra & Roychoudhury, 2020; Fletcher et al., 2000; Kamran et al., 2020). As stress conditions such as drought favour the production of oxygen radicals (Anjum et al., 2017; Jain et al., 2019; Nayyar & Gupta, 2006), their detoxification can be one reason for the stress-protecting ability of triazoles.

Drought stress in general restricts plant water use, which could lead to improved WUE (Davies et al., 2002; Liu et al., 2005; Passioura, 1996). Apart from overall water consumption, the distribution of available water throughout the entire growth period is decisive for yield development. Excessive vegetative growth can aggravate water limitations by using too much water before flowering (Passioura & Angus, 2010). However, sufficient water availability during the reproductive period is particularly important for kernel set and yield performance (Yang & Grassini, 2014). Under drought as well as under salt stress, reduced kernel setting as an important yield determinant for cereals was identified (Hütsch et al., 2015, 2014; Jung et al., 2017; Schubert et al., 2009; Setter & Parra, 2010; Zinselmeier et al., 1995), which can be partly compensated by an increased kernel weight (Hütsch et al., 2015, 2014; Jung et al., 2017; Schubert et al., 2009). However, a high grain yield can only be achieved with high kernel numbers. The smaller PAC-treated maize plants of our previous studies showed a significantly reduced water consumption in comparison to the untreated control (Hütsch & Schubert, 2018, 2021a). Consequently, the water-use efficiency ($WUE_{grain}$) was improved by 20% around the time of flowering and kernel setting. The determination of physiological mechanisms associated with the ability to maintain high kernel numbers under stress is of large concern. More emphasis on improving WUE with the aim of achieving better drought resistance is needed (Duvick, 2005).

In the present study, a container experiment was conducted with two maize cultivars, Galactus and Fabregas. At growth stage V8, the plants were treated once with PAC and concomitantly reduction in the soil water content commenced until 30–35% of the maximum water-holding capacity (WHC) were achieved. The plants were exposed to this drought conditions for 3 weeks bracketing silking, and cultivated until harvest at physiological maturity. Our investigations focused on the following hypotheses: with PAC application to maize plants facing drought stress during flowering, (a) grain yield can be improved due to a better kernel set, (b) maize plants show better root growth, and (c) water-use efficiency is increased during this critical period around flowering.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant cultivation, PAC application, and measurements during growth

The experiment was conducted at the experimental station of the Institute of Plant Nutrition in Giessen (50°35′53.30´´N, 8°40′1.56″ E) during the vegetation period of 2021. Maize plants (*Zea mays* L., cv. Galactus and cv. Fabregas) were cultivated according to Hütsch and Schubert (2021a, 2021b, 2022a) using the container technique; 120 L plastic containers were filled with 140 kg of a Luvisol subsoil (strong sandy loam: 18.9% clay, 28.2% silt, 52.9% sand; $N_{min}$: 0.8 mg N $kg^{-1}$, CAL-P: 10.3 mg P $kg^{-1}$, CAL-K: 88.5 mg K $kg^{-1}$; pH ($CaCl_2$) 5.6 prior to liming). The air-dry soil was mixed with $CaCO_3$ (2.5 g $kg^{-1}$ soil; pH ($CaCl_2$) 7.4 after liming) and filled into the containers in four layers: three layers with 30 kg soil moistened with 3 L deionized water each, and a topsoil layer (approx. 0–30 cm) which was fertilized with 40 g compound fertilizer ("Blaukorn") per container, consisting of 4.8 g N, 2.1 g P, 5.6 g K, 0.5 g Mg, 2.4 g S, 0.004 g Zn, and 0.008 g B. Additionally, 0.32 g Zn, 0.16 g Cu, and 0.08 g Mn were applied per container. The topsoil layer was moistened with 5 L deionized water. Four times during the vegetation period, additional fertilizer was applied (10 g "Blaukorn" per container), on 16 June, 28 June, 8 July, and 19 July.

On 18 May, the maize cultivars Galactus and Fabregas were sown. These two cultivars were chosen because in a screening experiment they showed the least negative impact of PAC application on grain yield (Hütsch & Schubert, 2021a). Seventeen days after sowing (DAS), the number of plants was reduced from nine to four per container. Water content of the soil was adjusted to 60% of the maximum water-holding capacity (WHC), which was kept for the treatment with optimal water supply throughout the entire growth period. For the determination of 100% WHC, a container filled with 140 kg air-dry soil was put into a water basin enabling water to infiltrate through holes at the container bottom until reaching the soil surface. The container was then removed from the water basin and let drain freely until weight constancy, which resembles the amount of water held in the soil against the gravity. This amount equals 100% WHC, and accordingly the value for any desired percentage of max. WHC can be calculated. In addition to the weight of container, soil and water, during vegetation increasing amounts of plant material were also accounted for. Starting with 1 July, water supply to half of the containers was slowly reduced until 30% of max. WHC was reached on 8 July. Thereafter, the water content of this treatment was adjusted to 30/35% of max. WHC until 28 July, when water supply was stepwise increased reaching again 60% of max. WHC on 3 August (2 weeks after first silk appearance). Thus, the maize plants were prone to drought stress of 30–35% of max. WHC for 3 weeks. During the entire vegetation period water content was adjusted to the desired value by water applications at least twice daily, and water supply was recorded for each container. With this experimental set-up, plant roots could exploit a rather large soil volume (120 L per container, soil depth approx. 80 cm, soil surface area 0.16 $m^2$). The plants grew in a vegetation hall under natural light conditions. The average daily temperature during the vegetation period ranged from 13°C to 31°C with a mean of 21.8 ± 0.3°C. The containers were set up in a completely randomized design and their position was changed at least once a week.

In addition to the two treatments of water supply, each maize genotype received three dosages of the plant growth regulator paclobutrazol (0, 2, 3 mg PAC per plant). On 30 June (43 days after sowing, DAS) between 12 and 1 p.m., PAC was applied at stage

V8, when the collar of the 8th leaf was visible in 91% of all plants, and 4% had already reached V9. This rather late growth stage was chosen to avoid negative effects on kernel primordia initiation and thus potential kernel number. Both cultivars received two dosages: 2 and 3 mg a.i. (active ingredient) PAC per plant. For this purpose, 34.94 and 52.42 mg PAC (22.9% w/w) were dissolved in 1 L of deionized water and poured onto the soil surface of the respective container, which resulted in the desired application of 2.0 and 3.0 mg a.i. PAC per plant. All solutions were prepared fresh in the morning of the application day. To ensure fast uptake of the chemicals by the plant roots, no water was applied to the containers on this day. The soil of the control treatment (0 mg PAC) received 1 L deionized water only. With two maize cultivars, two water regimes, three PAC dosages, and four replicates, the experiment consisted of 48 containers in total.

Growth stages were determined on the following dates: 28 and 30 June, 7 and 14 July (41, 43, 50, 57 DAS respectively). Plant height (measured from the shoot base to the tip of the longest leaf) was monitored on 30 June, and 07, 14, 21, and 30 July (43, 50, 57, 64, and 73 DAS, respectively). On 73 DAS (30 days after application, DAA), leaf areas were also determined by measuring length and maximal width of each leaf blade (leaf area = 0.5 × length × width). For 1 week (22 to 28 DAA), transpiration rates were calculated by dividing the consumed volume of water per plant and per day during this time span by the mean leaf area. Evaporative water losses, which on average accounted for 5% of total water demand during this period, were subtracted prior to calculation of transpiration.

From 17 July until 30 July, production of fresh pollen and start of silking were recorded for each plant daily at peak pollen shed (10 to 11 a.m.). From these data, the duration of pollen shed and the anthesis-silking interval ASI (start silking minus start pollen shed) were determined. For better comparisons of grain yield per plant and of yield components, such as single kernel weight and kernel number per cob, tillers were removed immediately after appearance. Axillary branches were not produced. Additional cobs on the main culm were not removed, as the effect of PAC application on this trait was evaluated. Start of senescence (yellowing of older leaves, appearance of red-coloured culms) was also recorded. Insecticides against *Oscinella frit* L. and European corn borer were applied when required.

## 2.2 | Harvest, root sampling, and analyses of plant material

For each maize cultivar, water supply treatment, and PAC application rate four containers with four plants each were harvested at physiological maturity, 139 and 140 DAS (4 and 5 October). Plant height, straw dry mass per container, cob dry mass per plant, cob length, kernel dry mass (80°C drying), kernel number per cob, and single kernel weight were determined. To determine various root parameters (fresh mass, dry mass, length, surface area), after harvest soil cores were collected in the centre of each container of the 0 and 3 mg PAC treatments. With an auger of 7.6 cm diameter, the soil was sampled in layers until the container-base was reached. After each layer was taken, the depth of the hole was measured. The soil cores were stored in plastic bags at 5°C until the samples were soaked and roots were carefully separated from soil and additional organic material in several sieving steps (smallest diameter of sieve pores: 1 mm) with cold water. Fresh mass was recorded prior to covering the roots with fixing solution (9: 1 mixture of 70% ethanol and 100% acetic acid) to prevent microbial decomposition. Root length and root surface area per sample were determined using the WinRhizo LA2400 Scanner (Regent Instruments Inc.). The root dry mass was recorded after drying at 80°C. Normalized root parameters were calculated in 10 and 30 cm increments, under the assumption that the core samples were representative of root distribution throughout the whole container (Selzer & Schubert, 2021).

The dried grain, straw, rachis, and root materials were milled to fine powder and dry-ashed. Potassium concentrations were determined using atomic absorption spectrometry, and phosphorus (P) concentrations were determined colorimetrically. Total N concentrations were measured using an elementary analyser (Unicube® trace, Elementar Analysensysteme GmbH, Langenselbold, Germany). Sample digestion was carried out via catalytic combustion at a temperature of 950°C, with the nitrogen-containing components in the sample forming $N_2$ which was measured with thermal conductivity detection. For the determination of the nutrient content per plant, which reflects net nutrient uptake of the biomass, the nutrient concentrations (mg g$^{-1}$ dry matter) were multiplied with the corresponding plant dry weights.

## 2.3 | Calculation of efficiency parameters and statistical analysis

Harvest index (HI), water-use efficiency of grain ($WUE_{grain}$), and nutrient-utilization efficiency of grain ($NutrientUtE_{grain}$) were calculated according to the following equations:

$$\mathrm{HI} = \text{Grain yield} / \text{Total above} - \text{ground biomass at physiological maturity} \quad (1)$$

$$\mathrm{WUE}_{grain} = \text{Grain dry matter} / \text{Total water consumption} \quad (2)$$

$$\mathrm{WUE}_{grain}\ \text{during silking} = \text{Grain dry matter} / \text{Water consumption during 5 days around silking (silking} \pm 5\ \text{days)} \quad (3)$$

$$\mathrm{NutrientUtE}_{grain} = \text{Grain dry matter} / \text{Nutrient content of total above} - \text{ground shoot biomass at physiological maturity} \quad (4)$$

Means ± standard errors (*SE*) were calculated from four replicates per growth regulator treatment, water supply (optimal vs. drought), and maize cultivar. A two-way ANOVA (factors: growth regulator treatment and water supply) was performed using *Rstudio* (Maintainer: Posit PBC; open source; Horton & Kleinman, 2015).

For the root parameters, a linear model was used prior to statistical evaluation, as some data were not balanced. Multiple comparisons of means were conducted for shoot and root data using students-*t*-test. *p*-values for multiple comparisons were adjusted using the *fdr*-method (Benjamini & Hochberg, 1995). Statistically significant differences are indicated using letter codes according to Piepho (2004) with a significance level of $\alpha = 5\%$ ($p < 5\%$). Differences between the two cultivars were not statistically evaluated.

## 3 | RESULTS

In the description of the results, at first effects of PAC application are pointed out for each maize cultivar and water supply. Subsequently, the impact of drought stress is described comparing its effects for each cultivar and PAC treatment. The well-watered treatment is named "control" treatment in comparison to drought stress; among the three PAC dosages 0, 2, and 3 mg PAC, the 0 mg PAC treatment is named "untreated".

### 3.1 | Vegetative plant growth and development, time of flowering

Both maize cultivars, either with optimal water supply or three weeks of drought stress during flowering, showed significant decreases in plant height after PAC application, and water limitation caused additional shortening of the plant shoots (Table 1). The total leaf area, determined 2 days after termination of drought, was unaffected by PAC treatment and water supply (Table 1). The transpiration rate, determined during the drought period, was unaffected by PAC, yet significantly reduced under water-limited conditions (Table 1). Six days after termination of drought, all plants looked healthy and green without any chlorosis or necrosis (Figure 1). PAC treatment delayed onset of senescence by approximately 4 to 5 days, whereas drought stress accelerated it slightly.

The tassels of drought-stressed maize plants were generally shorter with less branches than those of well-watered plants. Start and duration of pollen shed of both cultivars were mostly unaffected by PAC treatment and water supply (Table 2). Start of silking was significantly delayed under drought stress, causing significant increases in ASI in comparison to optimal water supply. For Fabregas, sporadic significant PAC effects occurred, resulting in faster silk appearance (optimal water supply with 2 mg PAC) and shorter ASI (drought with 2 mg PAC; Table 2).

### 3.2 | Yield determinants and harvest index

At maturity, all plants had produced one kernel-carrying cob (Figure 2). Untreated Galactus and Fabregas plants (0 mg PAC) with optimal water supply generally showed good kernel development over the entire cob. After PAC application, occasionally cob length was reduced and kernel abortion occurred in the apical part of some cobs. With drought stress, the cobs were shorter, showed reduced kernel setting at the tips more frequently, and in some cases, kernel distribution was scattered throughout the cob (Figure 2).

Grain yield was unaffected by PAC application, whereas drought stress caused significant decreases by 15% on average of both cultivars (Figure 3a). With increasing PAC dosage, Galactus showed a stepwise decrease in straw yield, yet with Fabregas 2 mg and 3 mg PAC resulted in a similar decrease compared to 0 mg PAC. Drought stress significantly reduced straw yield in both cultivars (Figure 3b). With drought-stressed Galactus plants, a significant increase of the harvest index by 11% was achieved after application of 3 mg PAC, and water limitation itself showed no effect on the harvest index (Figure 3c).

**TABLE 1** Plant height at 30 DAA, total leaf area at 30 DAA, and transpiration rate (calculated for the timespan 22 to 28 DAA) of two maize cultivars under optimal water supply (control) and drought stress conditions, with application of the plant growth regulator paclobutrazol (PAC); data show means of four replicates ± SE; significant differences ($p < 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas); values which share at least one letter do not differ significantly; DAA = days after application of PAC.

| Maize cultivar | Water supply | PAC treatment | Plant height (cm) 30 DAA | Leaf area (cm$^2$ plant$^{-1}$) 30 DAA | Transpiration rate (mL cm$^{-2}$ d$^{-1}$) 22 to 28 DAA |
|---|---|---|---|---|---|
| Galactus | Optimal | 0 mg | 321 ± 3 a | 4474 ± 52 a | 0.112 ± 0.005 a |
| | | 2 mg | 296 ± 8 b | 4585 ± 31 a | 0.122 ± 0.009 a |
| | | 3 mg | 294 ± 3 b | 4544 ± 39 a | 0.104 ± 0.008 a |
| | Drought | 0 mg | 273 ± 4 c | 4557 ± 78 a | 0.078 ± 0.006 b |
| | | 2 mg | 265 ± 6 c | 4389 ± 96 a | 0.079 ± 0.004 b |
| | | 3 mg | 244 ± 3 d | 4258 ± 112 a | 0.079 ± 0.003 b |
| Fabregas | Optimal | 0 mg | 332 ± 6 A | 3829 ± 86 A | 0.142 ± 0.004 A |
| | | 2 mg | 314 ± 4 AB | 3647 ± 49 A | 0.127 ± 0.015 AB |
| | | 3 mg | 308 ± 9 B | 3860 ± 73 A | 0.122 ± 0.007 AB |
| | Drought | 0 mg | 283 ± 3 C | 3552 ± 103 A | 0.105 ± 0.004 BC |
| | | 2 mg | 261 ± 3 D | 3593 ± 134 A | 0.081 ± 0.003 C |
| | | 3 mg | 245 ± 8 D | 3674 ± 30 A | 0.088 ± 0.003 C |

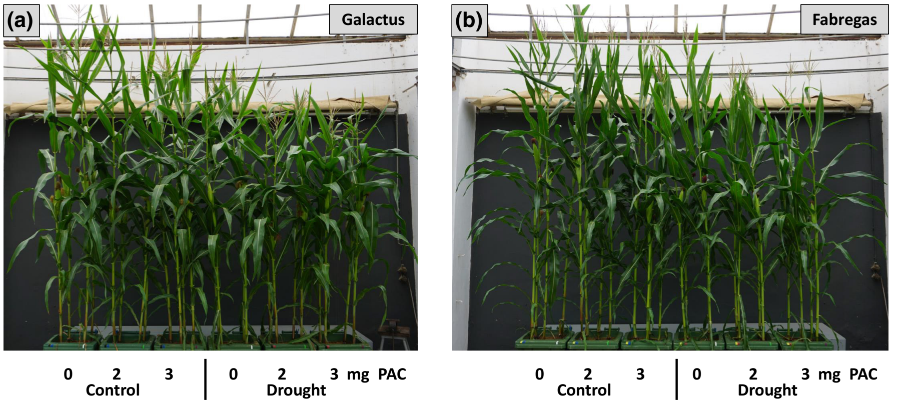



**FIGURE 1** Effect of the plant growth regulator paclobutrazol (PAC) on the development of maize cultivars Galactus and Fabregas, grown under well-watered (control) conditions or with drought stress during flowering; the photos were taken 77 days after sowing (DAS), 34 days after PAC application (DAA), and 6 days after termination of drought stress; the PAC dosages were 0, 2, 3 mg a.i. per plant.

**TABLE 2** Start and duration of pollen production, start of silking, and anthesis-silking interval (ASI; start silking minus start pollen production) of two maize cultivars under optimal water supply (control) and drought stress conditions, with application of the plant growth regulator paclobutrazol (PAC); data show means of four replicates ± SE; significant differences ($p < 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas); values which share at least one letter do not differ significantly.

| | | | Pollen production | | Silking | Anthesis-Silking interval |
|---|---|---|---|---|---|---|
| Maize cultivar | Water supply | PAC treatment | Start (DAS) | Duration (d) | Start (DAS) | ASI (d) |
| Galactus | Optimal | 0 mg | 63.6 ± 0.2 a | 3.9 ± 0.3 a | 64.0 ± 0.3 b | 0.4 ± 0.2 b |
| | | 2 mg | 63.4 ± 0.6 a | 4.8 ± 0.5 a | 63.1 ± 0.5 b | −0.3 ± 0.2 b |
| | | 3 mg | 63.6 ± 0.2 a | 4.7 ± 0.1 a | 63.4 ± 0.3 b | −0.2 ± 0.1 b |
| | Drought | 0 mg | 64.0 ± 0.2 a | 4.1 ± 0.2 a | 66.2 ± 0.4 a | 2.2 ± 0.4 a |
| | | 2 mg | 64.3 ± 0.4 a | 4.1 ± 0.2 a | 65.8 ± 0.2 a | 1.4 ± 0.2 a |
| | | 3 mg | 64.2 ± 0.1 a | 4.7 ± 0.2 a | 65.7 ± 0.2 a | 1.5 ± 0.3 a |
| Fabregas | Optimal | 0 mg | 63.1 ± 0.2 AB | 4.6 ± 0.4 A | 64.4 ± 0.2 BC | 1.3 ± 0.1 BC |
| | | 2 mg | 62.3 ± 0.2 B | 4.3 ± 0.2 A | 63.1 ± 0.2 D | 0.8 ± 0.1 C |
| | | 3 mg | 62.8 ± 0.3 AB | 4.1 ± 0.2 A | 63.5 ± 0.3 CD | 0.7 ± 0.1 C |
| | Drought | 0 mg | 64.2 ± 0.5 A | 3.6 ± 0.2 A | 66.6 ± 0.7 A | 2.4 ± 0.4 A |
| | | 2 mg | 64.2 ± 0.6 A | 4.3 ± 0.2 A | 65.4 ± 0.5 AB | 1.3 ± 0.1 BC |
| | | 3 mg | 63.9 ± 0.4 A | 3.4 ± 0.5 A | 65.9 ± 0.3 A | 2.0 ± 0.5 AB |

Significant effects of PAC on kernel number were only observed for drought-stressed Galactus plants with an increase due to 3 mg PAC compared to 0 mg PAC (Figure 4a). Water limitation caused significant decreases in kernel number in all cases. The increase in kernel number after PAC treatment of drought-stressed Galactus plants was accompanied by a significant decrease in single kernel weight, whereas no other PAC effects on kernel weight were observed (Figure 4b). Drought stress caused significant increases in single kernel weight of Galactus, whereas Fabregas was not affected (Figure 4b). PAC treatment caused significant reductions in cob length only for Fabregas, under control as well as under stress conditions, and water limitation decreased cob length in all cases (Figure 4c).

## 3.3 | Water consumption and water-use efficiency (WUE)

Water consumption, determined for the entire growth period, was significantly decreased by PAC in drought-stressed Fabregas plants with similar effects of 2 mg and 3 mg PAC compared to 0 mg PAC (Figure 5a). In all cases, drought stress significantly reduced total water consumption by 16% on average. When water consumption during silking was considered (start silking ±5 days), similar PAC effects were obtained, whereas the decrease due to drought was much stronger (28% on average; Figure 5b). PAC application to drought-stressed Fabregas plants caused significant improvements of water-use efficiency ($WUE_{grain}$) during silking, which in some cases was also increased due to drought stress itself (Figure 5c).

## 3.4 | Root dry matter, root length, and root surface

In the root studies, the 0 mg and 3 mg PAC treatments of both maize cultivars, grown either under well-watered (control) conditions or subjected to drought stress during flowering, were investigated. The root samples were collected immediately after harvest at physiological maturity. In the figures, the values for three depth increments down to the base of the container are given.

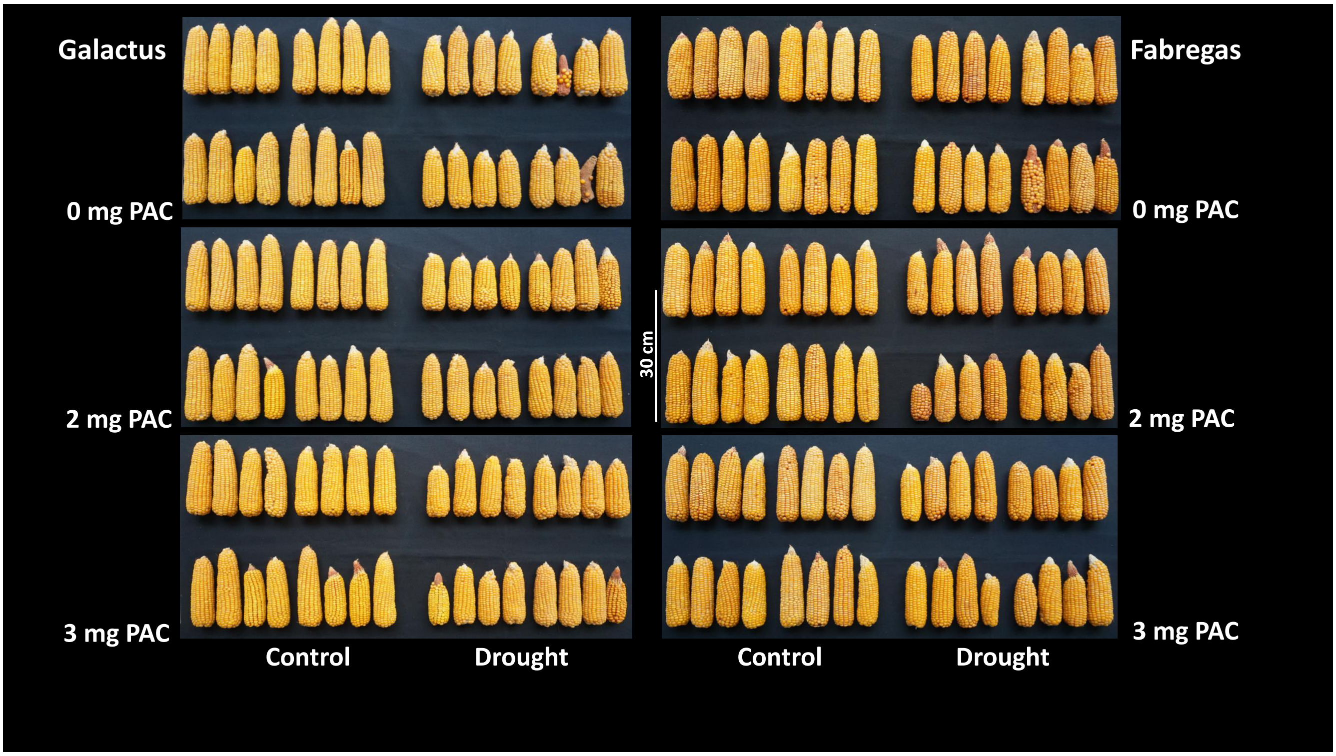



FIGURE 2 Effect of the plant growth regulator paclobutrazol (PAC) and different water supply (well-watered (control) conditions or drought stress during flowering) on cob development of maize cultivars Galactus and Fabregas at maturity (at 139/140 days after sowing (DAS)); the PAC dosages were 0, 2, 3 mg a.i. per plant.

The by far largest quantity of roots was found in the 0 to 30 cm soil layer (Figure 6). The root dry matter density of both cultivars varied somehow due to PAC treatment and water supply, however, no significant effects were observed throughout the soil profile (Figure 6a,b). Root length density of Galactus showed an increase in 0–30 cm due to drought stress combined with 0 mg PAC, whereas in the other depths and for Fabregas no treatment effects were found (Figure 6c,d). Also for root surface density, for both cultivars no significant effects of PAC treatment or water supply occurred (Figure 6e,f).

In Figure 7, root parameters are given on a per-plant basis. For both maize cultivars, root dry matter, root length, and root surface area showed no significant effects of PAC application or water supply. Combined with drought stress, PAC treatment tended to decrease these parameters (Figure 7). There were no significant effects of PAC or water supply on root-to-shoot ratios (results not shown).

## 3.5 | Nutrient (N, P, K) concentrations, contents, and utilization efficiencies

In the present growth regulator experiment, all maize plants were supplied with the same fertilizer amounts. Nitrogen concentrations in grain and straw were significantly increased under water limitation, and in the straw of drought-stressed Fabregas plants, PAC treatment resulted in significantly higher values compared to 0 mg PAC (results are not shown). The nutrient contents per plant can be considered as net uptake, although the values for the roots could be underestimated due to nutrient losses during the washing procedure for root preparation (see Materials and Methods). For both maize cultivars, the N contents of shoot and root were unaffected by PAC application and drought stress (Table 3). From the applied fertilizer-N (in total 2.4 g N per plant), which was the only available N source, the plants took up 81% on average. This means luxurious N consumption for the smaller plants with growth reductions caused by drought, PAC or both treatments. Consequently, N-utilization efficiency ($NUtE_{grain}$) was significantly decreased under drought stress resulting from grain yield depressions combined with similar N uptake (Table 3). $NUtE_{grain}$ was mostly unaffected by PAC application (Table 3).

For Galactus, neither PAC treatment nor water limitation showed a significant impact on P contents of shoot and root, which was also true for Fabregas roots (Table 3). However, P content in Fabregas shoot was significantly decreased due to drought stress with no effect of PAC. The P-utilization efficiency ($PUtE_{grain}$) was improved with PAC treatment of drought-stressed Galactus plants (Table 3). Water limitation itself caused a significant decrease in $PUtE_{grain}$ only for Galactus with 0 mg PAC (Table 3). The K content in roots was unaffected by PAC treatment and water supply, whereas in shoots it decreased under drought stress, and for Fabregas under well-watered conditions PAC caused a significant increase (Table 3).

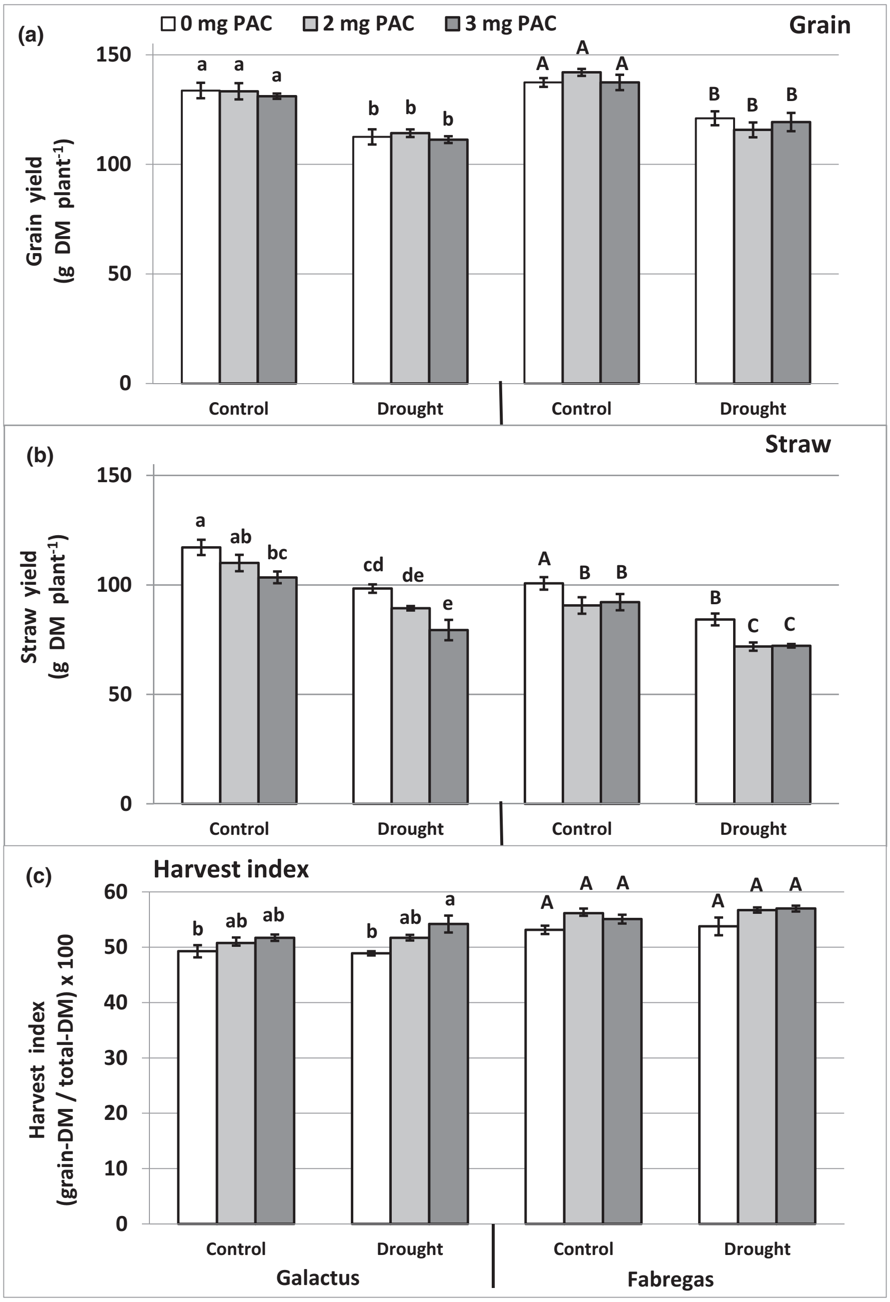



**FIGURE 3** Grain dry matter yield (a), straw dry matter yield (b) and harvest index (c) of two maize cultivars under optimal water supply (control) and drought stress conditions, with application of the plant growth regulator paclobutrazol (PAC); columns with error bars show means of four replicates ± *SE*; significant differences ($p < 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas); columns which share at least one letter do not differ significantly.

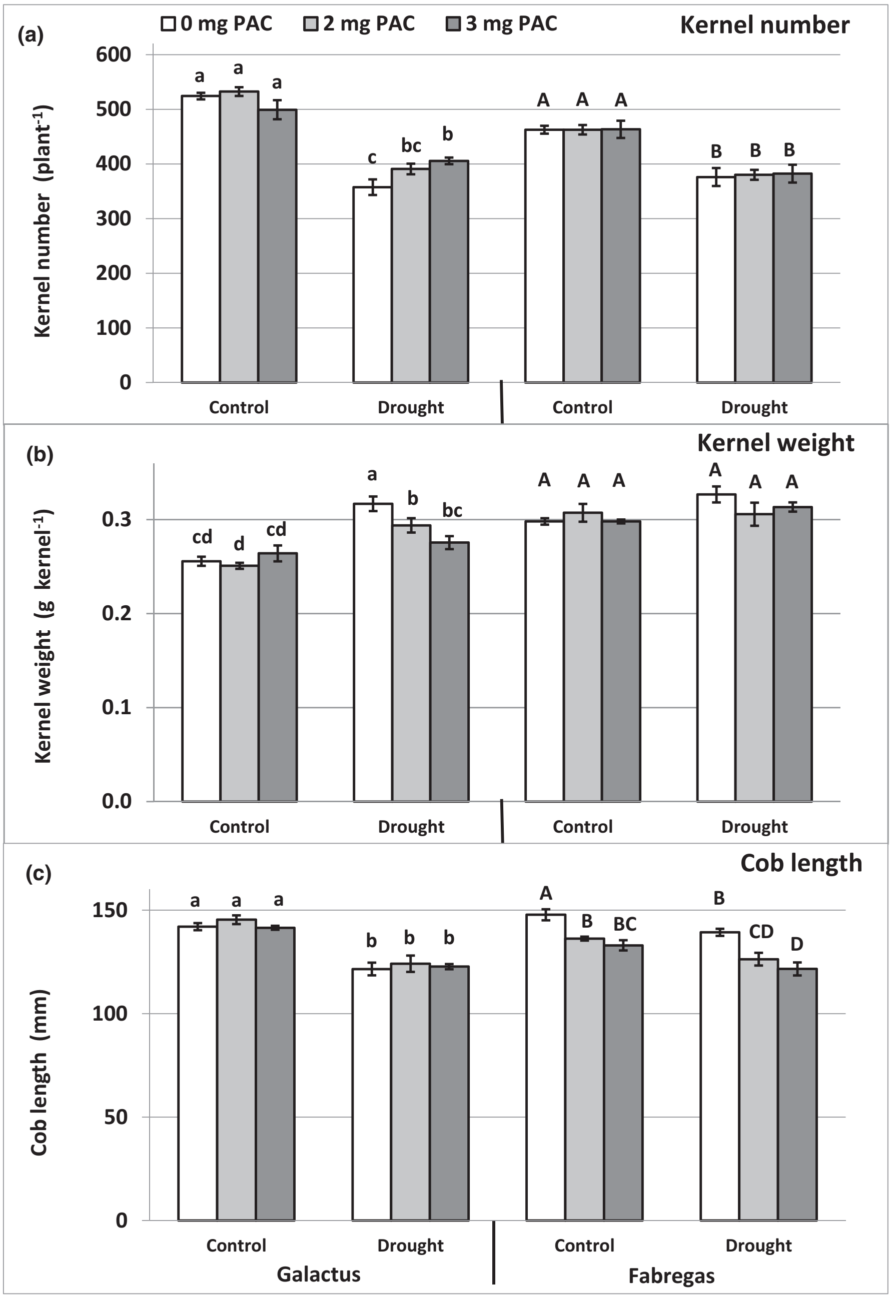
K-utilization efficiency ($KUtE_{grain}$) was affected neither by PAC nor by water supply (Table 3).

## 4 | DISCUSSION

### 4.1 | Grain yield was not improved with PAC application to drought-stressed maize plants

For both tested maize cultivars, grain yield at physiological maturity was unaffected by PAC application under optimal water supply as well as with drought stress during flowering (Figure 3a). Although for stressed Galactus plants an increase in kernel number per plant was achieved after PAC treatment, simultaneously single kernel weight, the other important determinant of maize grain yield, decreased (Figure 4a,b). In several studies, stress protection of plants, observed after treatment with triazole compounds, was related to improved antioxidant activity leading to less damage of plant cells by reactive oxygen species, and thus maintenance of high photosynthetic rates (Bayat & Sepehri, 2012; Chandra & Roychoudhury, 2020; Fletcher et al., 2000; Kamran et al., 2020). A positive effect of a higher availability of carbon assimilates, needed for synthesis and energy purposes, will only cause increases in grain yield if its production is source-limited. This could occur when plants suffer from strong stress conditions causing intensified decreases in vegetative shoot growth to the point of necroses and thus dying of formerly photosynthetically active leaves. In the present study, the soil water content was adjusted to achieve between 30% and 35% of max. WHC at least

FIGURE 4 Kernel number (a), single kernel weight (b) and cob length (c) of two maize cultivars under optimal water supply (control) and drought stress conditions, with application of the plant growth regulator paclobutrazol (PAC); columns with error bars show means of four replicates ± *SE*; significant differences ($p < 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas); columns which share at least one letter do not differ significantly.




twice daily, and thus, drought stress intensity can be considered as medium. Decreases in straw yield due to drought (Figure 3b) resulted mainly from reductions in plant height (Table 1) and thus culm biomass, whereas the area of green leaf blades was unaffected by water stress (Table 1), making decreases in photosynthetic activity unlikely.

Apart from the assimilate supply in leaves, their availability in developing kernels is even more important for grain yield production. In our previous studies, conducted under drought or saline conditions, the sucrose availability in maize kernels, harvested 0 or 2 days after pollination, was either unchanged or significantly increased in comparison to the unstressed control (Hütsch et al., 2015, 2020; Jung et al., 2017). Therefore, kernel establishment and development were not source-limited. Furthermore, the concentrations of the hexoses glucose and fructose, which are the transport metabolites from the mother plant to the developing kernels (Hütsch & Schubert, 2017), were also enhanced in the stressed plants. Under these conditions, sink limitation was responsible for grain yield reductions due to stress conditions. Sink strength can be restricted either by reduced sink activity or a smaller sink capacity (Ho, 1988). Around the critical period of kernel setting, the enzymes acid invertase and plasma membrane $H^+$-ATPase mainly determine sink activity, and their inhibition restricts the hexose transport into the kernels and finally leads to kernel abortion (Hütsch & Schubert, 2017, 2022b; Jung et al., 2017). In addition to forced kernel abortion under stress conditions, insufficient fertilization resulting from retarded silking and an extension of the anthesis-silking interval ASI (Table 2) contributed

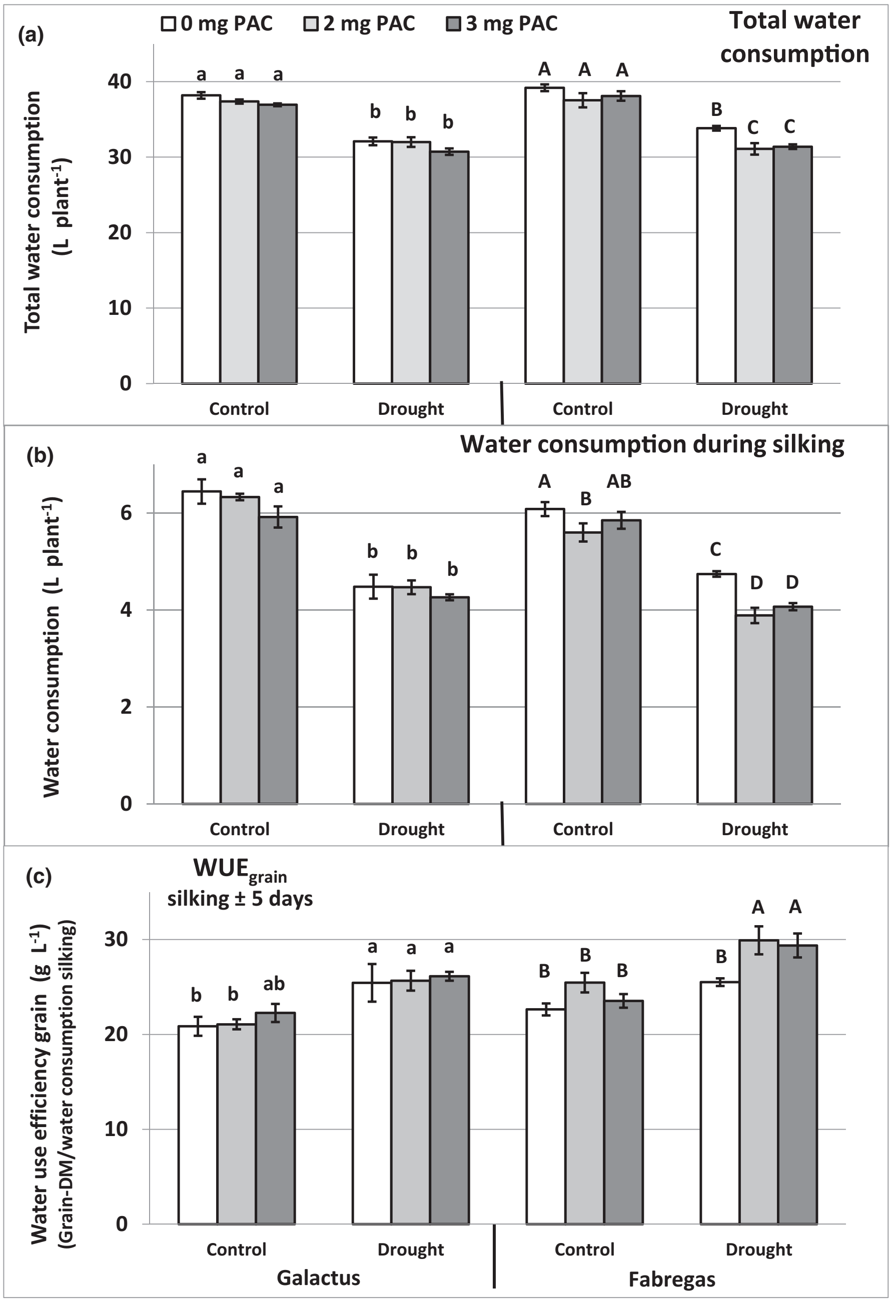



**FIGURE 5** Total water consumption (a), water consumed during silking ±5 days (b) and $WUE_{grain}$ for the time during silking ±5 days (c) of two maize cultivars under optimal water supply (control) and drought stress conditions, with application of the plant growth regulator paclobutrazol (PAC); columns with error bars show means of four replicates ± *SE*; significant differences ($p<5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas); columns which share at least one letter do not differ significantly.

to the reduced kernel number (Figure 4a). Less kernels and shorter cobs (Figure 4a,c) diminished sink capacity and thus sink strength.

It can be concluded that grain yield performance of drought-stressed maize plants was not source-limited but sink-limited. Consequently, even if PAC treatment improved antioxidant activity and photosynthesis, an effect on grain yield is unlikely due to sufficient availability of assimilates.

## 4.2 | Root growth was not increased with PAC application to drought-stressed maize plants

In several studies with triazole treatment of drought-stressed maize plants, an increase in root parameters such as biomass, length, surface area, and diameter was found (Kamran, Wennan, et al., 2018; Urfan et al., 2022; Wang et al., 2022). In some studies,

**FIGURE 6** Root dry matter density (a, b), root length density (c, d), and root surface density (e, f) of the maize cultivars Galactus (left side) and Fabregas (right side), grown under optimal water supply (control) and drought stress conditions, with application of the plant growth regulator paclobutrazol (0 or 3 mg a.i. PAC per plant); data were extracted from a linear model, bars represent means, lines represent *SE*; significant differences ($p<5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas); if bars share at least one letter, there is no significance; *p*-values were adjusted using the *fdr*-method; letters can only be compared within one soil depth; hatched bars cannot be compared.

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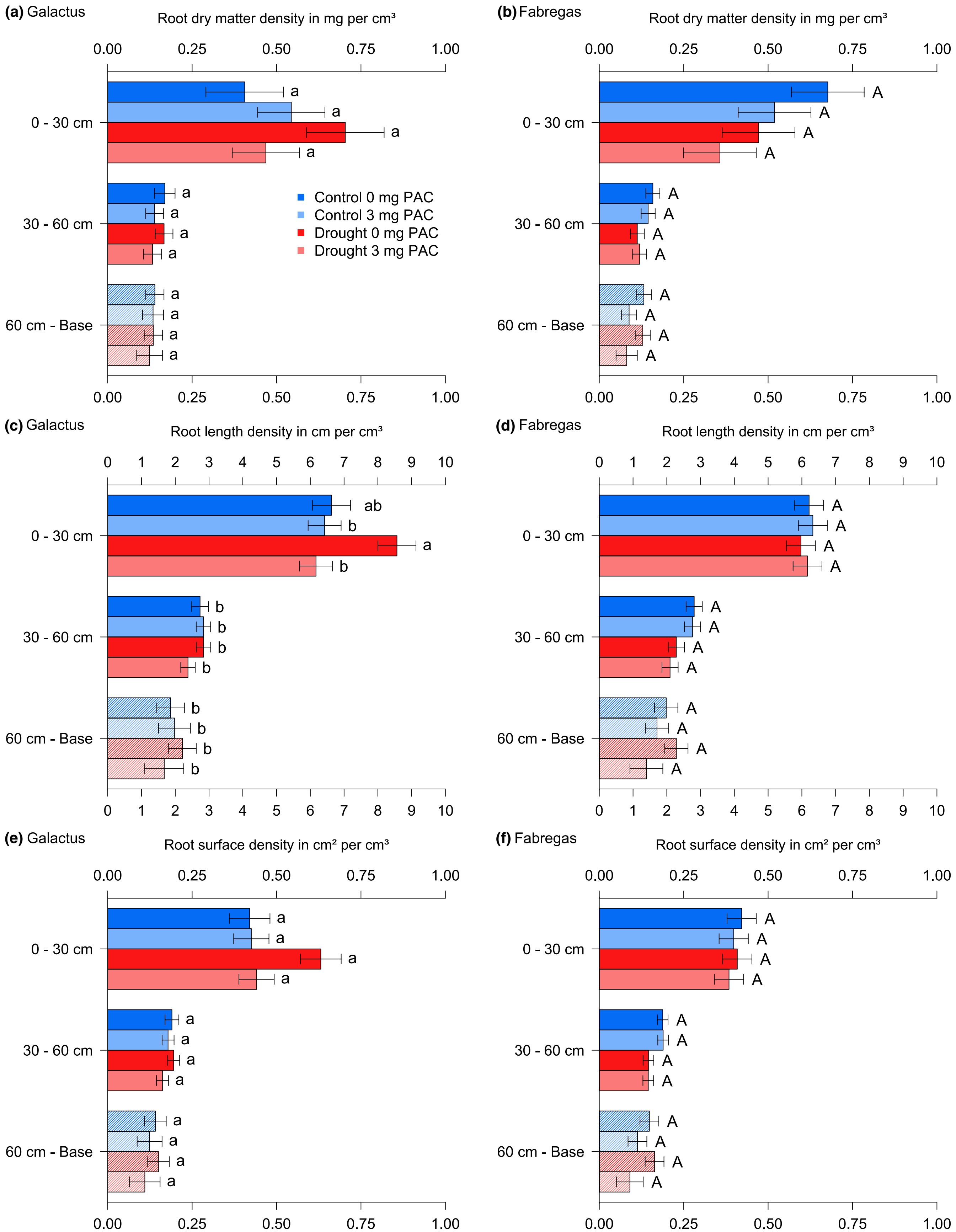

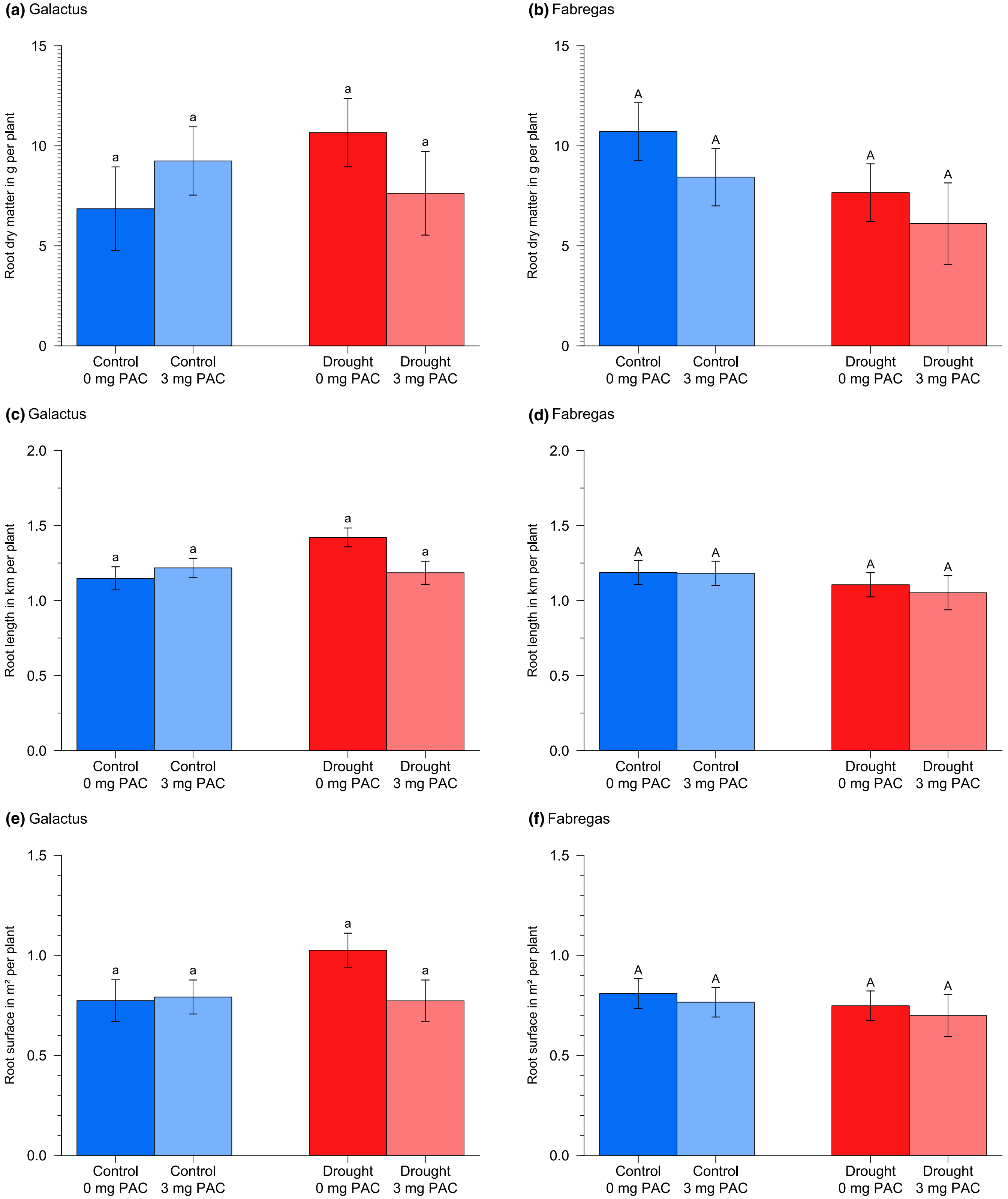


**FIGURE 7** Root dry matter (a, b), root length (c, d), and root surface (e, f) of the maize cultivars Galactus (left side) and Fabregas (right side), grown under optimal water supply (control) and drought stress conditions, with application of the plant growth regulator paclobutrazol (0 or 3 mg a.i. PAC per plant); data were extracted from a linear model, bars represent means, lines represent *SE*; significant differences ($p < 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas); if bars share at least one letter, there is no significance; *p*-values were adjusted using the *fdr*-method.

**TABLE 3** Nitrogen (N), phosphorus (P), and potassium (K) contents in total above-ground biomass (shoot) and root of two maize cultivars under optimal water supply (control) and drought stress conditions, with application of the plant growth regulator paclobutrazol (PAC), and the respective nutrient-utilization efficiencies $NUtE_{grain}$, $PUtE_{grain}$, $KUtE_{grain}$ (grain-DM/total nutrient content); data show means of four replicates; significant differences ($p < 5\%$) within each cultivar are indicated by small (Galactus) or capital letters (Fabregas); values which share at least one letter do not differ significantly; nd = not determined.

| Maize cultivar | Water supply | PAC treatment | N content (mg N plant$^{-1}$) Shoot | N content Root | $NUtE_{grain}$ (g g$^{-1}$) | P content (mg P plant$^{-1}$) Shoot | P content Root | $PUtE_{grain}$ (g g$^{-1}$) | K content (mg K plant$^{-1}$) Shoot | K content Root | $KUtE_{grain}$ (g g$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Galactus | Optimal | 0 mg | 1847 a | 52.5 a | 72.4 a | 324.5 a | 4.1 a | 412.0 a | 2628 abc | 8.8 a | 50.9 a |
| | | 2 mg | 1911 a | nd | 69.8 a | 341.8 a | nd | 391.6 a | 2795 a | nd | 47.7 a |
| | | 3 mg | 1860 a | 65.1 a | 70.5 a | 310.1 a | 5.6 a | 423.3 a | 2734 ab | 16.5 a | 48.0 a |
| | Drought | 0 mg | 1919 a | 85.8 a | 58.9 c | 337.4 a | 6.6 a | 334.5 b | 2536 bc | 18.8 a | 44.5 a |
| | | 2 mg | 1815 a | nd | 62.9 b | 295.5 a | nd | 389.9 a | 2430 cd | nd | 47.3 a |
| | | 3 mg | 1813 a | 66.1 a | 61.4 bc | 284.0 a | 4.8 a | 392.6 a | 2251 d | 15.5 a | 49.6 a |
| Fabregas | Optimal | 0 mg | 1935 A | 68.5 A | 71.0 AB | 332.2 A | 5.2 A | 413.8 A | 2313 B | 15.2 A | 60.2 A |
| | | 2 mg | 1913 A | nd | 74.3 A | 330.9 A | nd | 429.2 A | 2584 A | nd | 55.0 A |
| | | 3 mg | 1896 A | 54.1 A | 72.6 AB | 349.2 A | 4.2 A | 393.7 A | 2619 A | 12.7 A | 52.6 A |
| | Drought | 0 mg | 1813 A | 57.6 A | 66.8 BC | 281.8 B | 4.6 A | 430.2 A | 2262 B | 11.3 A | 53.6 A |
| | | 2 mg | 1886 A | nd | 61.5 C | 266.9 B | nd | 435.2 A | 2213 B | nd | 52.3 A |
| | | 3 mg | 1882 A | 53.1 A | 63.4 C | 290.3 B | 3.9 A | 412.0 A | 2109 B | 10.5 A | 56.6 A |

maize seedlings were investigated, and apart from early vegetative growth, no information is available for generative growth or grain yield at maturity (Barnes et al., 1989; Wang et al., 2022). Experiments considering the entire maize plant ontogenesis were either conducted in small pots (7 kg soil per plant; Urfan et al., 2022), or under semiarid field conditions with irregular rainfall events to predominantly drought-stressed plants (Kamran, Wennan, et al., 2018). The cultivation of maize plants in a small soil volume has the disadvantage that it is difficult to keep the huge plants well watered (control conditions) during flowering until physiological maturity. Temporary water deficits also under "control" conditions are likely to occur. In our container studies, this was avoided as four maize plants could exploit a volume of 120 L containing 140 kg soil. In addition, a large soil volume is a prerequisite for reliable root studies.

One explanation for improvements of root growth after triazole application to stressed plants is an increased partitioning of assimilates from the smaller shoots to the roots due to the decreased demand in the shoots (Fletcher et al., 2000). This might be important during vegetative growth, but with start of kernel development, the maize cob is the strongest sink for assimilates and translocation to the roots is of minor importance. This is strengthened by the result that at maturity the dry matter per plant of root and cob was approximately 10 g and 130 g, respectively (Figures 3a and 7a), and root biomass accounted for only about 4% of total plant biomass. Considering these aspects, in our study with drought stress during flowering, enhanced root growth due to PAC application was unlikely to occur.

Another point of discussion for triazole effects on root growth under stress conditions is the interference with plant hormones. The primary target of triazoles is the inhibition of biosynthesis of gibberellins causing reduced plant extension growth. This effect causes reductions definitely in shoot growth (e.g. Hütsch & Schubert, 2018, 2021a, 2021b, 2022a; Kamran, Cui, et al., 2018; Schluttenhofer et al., 2011), but why should, due to less gibberellins, an increase in root elongation occur at the same time? In fact, the opposite was observed in the very early study of Whaley and Kephart (1957), where application of gibberellic acid significantly stimulated root growth of certain maize genotypes.

Additionally, secondary hormone effects of triazoles were observed such as increased levels of abscisic acid (ABA) and cytokinins, and inhibition of ethylene biosynthesis (Ahmad et al., 2018, 2019; Desta & Amare, 2021; Fletcher et al., 2000; Wang & Lin, 1992). Gibberellins and ABA are both synthesized via the terpenoid pathway (Desta & Amare, 2021). When the biosynthesis of gibberellins is inhibited, more precursors are available to generate ABA molecules (Rademacher, 2000). Additionally, the degradation of ABA is inhibited by PAC as well (Desta & Amare, 2021). Therefore, in some studies, increases in endogenous ABA concentrations were found after PAC treatment to various crop species (Desta & Amare, 2021; Hauser et al., 1990; Mackay et al., 1990). ABA is considered to decrease cell extension and thus plant growth, although root growth stimulation due to ABA was also observed (Yan et al., 1992). Improved root growth would result in higher cytokinin levels, as root meristems are major sites of cytokinin biosynthesis (Binns, 1994). Cytokinins are primarily responsible for cell division and thus could by this means increase root growth. An inhibition of ethylene synthesis could be beneficial for root development, as under stress conditions, ethylene reduced cell proliferation at the root apical meristem and thus may contribute to impaired root growth (Cao et al., 2007; Street et al., 2015). Although some information on the role of each hormone in root development is available, knowledge about their simultaneous actions on the same process and their networking is limited (Sánchez-Romera & Aroca, 2020). Thus, discussion about hormone effects on triazole-treated roots is still very speculative and further profound studies are needed.

### 4.3 | Water-use efficiency ($WUE_{grain}$) was improved with PAC application to drought-stressed maize plants

In our previous container experiments with PAC application to well-watered maize plants, significant increases in $WUE_{grain}$ during the critical period around flowering were achieved (Hütsch & Schubert, 2018, 2021a, 2021b). For the maize cultivar Fabregas, under drought stress, this improvement could be confirmed (Figure 5c). This effect relied entirely on the decreased water consumption (Figure 5a,b), as grain yield was unchanged (Figure 3a). The respective maize plants are characterized by the smallest straw yield and thus biomass production (Figure 3b). The plants could afford to keep the stomata closed for longer periods, resulting in decreased transpirational water losses. This is also indicated in decreased transpiration rates, although the differences between 0 mg versus 2 mg and 3 mg PAC were not significant (Fabregas, drought; Table 1). It can be summarized that under water limitation, PAC can relieve stress intensity due to a more efficient use of the available water.

## AUTHOR CONTRIBUTIONS

**Birgit W. Hütsch:** Conceptualization; data curation; formal analysis; funding acquisition; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Lukas Kehm:** Data curation; formal analysis; investigation; methodology; validation; visualization; writing – review and editing. **Sven Schubert:** Conceptualization; methodology; resources; supervision; writing – review and editing.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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