sustainability

MDPI

Article

Exploring Local Maize Diversity for Increased Agricultural Sustainability: New Insights into Drought Stress Response and Recovery of Guinea-Bissau Landraces

Manuela Teixeira [1], Eduardo Feijão [1], Luís Catarino [2], Ana Rita Matos [1], Andreia Figueiredo [1] and Jorge Marques da Silva [1,*]

[1] Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; manuelaconceicao18@gmail.com (M.T.); emfeijao@fc.ul.pt (E.F.); armatos@fc.ul.pt (A.R.M.); aafigueiredo@fc.ul.pt (A.F.)

[2] Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; lmcatarino@fc.ul.pt

* Correspondence: jmlsilva@fc.ul.pt

Abstract: Landraces are rightfully known as the backbone of sustainable food production, particularly in areas experiencing significant environmental constraints. However, protecting landraces from genetic erosion and valuing their potential for plant breeding and sustainable food production requires in-depth understanding of their phenotypic traits. Two Guinea-Bissau landraces (GA, GV) and two elite cultivars (P98438, P0023) were subjected to drought stress for 7 (S1) and 12 (S2) days. After this period plants were rewatered (R). The relative water content (RWC) was unchanged in S1 and decreased in S2 in all genotypes. Chlorophyll *a* fluorescence parameters changed moderately in S1 and remarkably in S2, including on GA and GV plants, where a decrease of Fv/Fm and PI, and an increase in absorbed, trapped, and dissipated energy per reaction center, was found. P0023 plants showed the most contrasting behavior to Guinea-Bissau genotypes, presenting an increase in Fv/Fm and PI values and a decrease in the specific energy fluxes per reaction center (RC), whereas P9838 presented an intermediate behavior. Drought (S1 and S2) decreased the amount of chlorophyll (Chl.) and carotenoids in GA and GV plants. On the contrary, in the P0023, the only pigment to decrease with stress was Chl. *b*. Fatty acid (FA) analyses allowed for the identification of C16:0, C18:2, and C18:3 in larger amounts, and C14:0, C16:1*t*, C18:0, and C18:1 acids in lower abundance. Drought stress decreased C18:3, the double bond index, and the total FA (except for P0023 and GV in S2) and increased C14:0 and C16:0 acids. The expression of phospholipase D (PLD) was higher at S2. After recovery, PLD expression presented a notorious decrease on the Guinea-Bissau landraces. P0023 showed the smallest alterations after recovery, while Guinea's plants suffered more radical alterations leading to the conclusion that Guinea's landraces were more drought-sensitive and that their recovery rate was impaired.

Keywords: chlorophyll *a* fluorescence; fatty acids; phospholipase D; photosynthetic pigments

Citation: Teixeira, M.; Feijão, E.; Catarino, L.; Matos, A.R.; Figueiredo, A.; Marques da Silva, J. Exploring Local Maize Diversity for Increased Agricultural Sustainability: New Insights into Drought Stress Response and Recovery of Guinea-Bissau Landraces. *Sustainability* **2021**, *13*, 5441. https://doi.org/10.3390/su13105441

Academic Editors: Roberto Mancinelli, Filipa Monteiro, Mónica Sebastiana and Patrícia Vidigal

Received: 13 April 2021
Accepted: 9 May 2021
Published: 13 May 2021

1. Introduction

Maize (*Zea mays* L.) is a cereal that was domesticated in Central America and brought to Africa, Asia, and Europe by the Portuguese and the Spanish in the XVI century [1]. Nowadays it is the third most important grain crop, grown commercially in over 100 countries and used as a human staple food and for animal feed [2–4].

According to Santpoort [5], the maize produced in West Africa accounts for about one-third of the total harvested in the continent, and the modest yields obtained, ranging from 0.8 to 1.5 ton ha^{-1}, are related to the rainfall levels in the region. However, a trend to increasing production was noted in the last decades, mainly due to yield raising because of the use of improved seeds and, to a lesser extent, due to the access of farmers to inputs such as fertilizers, pesticides, and irrigation.

In Guinea-Bissau, West Africa, maize is not the staple food, but it has an important role in the food security of families in rural areas. The vernacular name of maize in Guinea-Bissau creole, midjo-basil, derived from milho-do-Brasil, reflects the origin of the crop. As it requires well-drained and fertile soils, it is cropped for self-consumption by small farmers in home gardens and areas around the villages in Ferralsols enriched by domestic debris or livestock manure, and it is often associated with other crops, such as cassava, groundnuts, beans, or tomato. In Guinea-Bissau, as all over the world, differences in agroecological conditions and agricultural practices between communities have resulted in the development of locally adapted varieties, known as landraces [6]. Maize landraces are still used there in local subsistence agriculture; albeit, these important constituents of agro-biodiversity continue to face the risk of depletion worldwide [7]. Implementing breeding programs for improved landraces is one of the strategies available to improve yield and yield stability in less favorable agricultural systems, with lower input levels, increasing agricultural sustainability and food security and sovereignty. In fact, landraces still support farming because of their stability, resistance to biotic and abiotic stresses, and end-product quality [8]. Furthermore, landraces may be useful to breed elite cultivars, which are obtained from a quite narrow germplasm pool and are mostly well fitted to high-input agriculture [9].

It is estimated that the yield of maize crops is diminished annually in many food-production regions because of excessive temperatures, rainfall distributions changes, and drought [2,10,11]. With the alarming increase of world population, expected to reach about nine billion by the end of 2050, a decrease in maize production will be a major problem in face of the increasing food requirements [12,13]. Therefore, it is extremely important to understand the effect of drought stress on maize to guarantee food supply.

Guinea-Bissau has a dry to sub-humid and hot climate and most agriculture production depends on these climate conditions to succeed. Maize has a short vegetative cycle of 80–90 days; thus, in regions with favorable conditions of rainfall, two crops can be grown per year. The first is sown in June or early July, at the beginning of the rains, and harvested in September or early October when rice, the main crop, is not yet mature, and the second is sown in September and harvested in December. Two annual harvests can be obtained in the Southwest and Bijagós Islands, but in the North and East of the country, only one annual harvest is obtained. There is no available data on maize yield in the country but, taking into account that the seeds sown are not improved for high production, as well as the rudimentary agricultural practices, virtually without inputs, the yield obtained must be below 1 ton ha^{-1} [14].

Climate change and continuous population growth poses serious threats to world food safety [15–18]. According to Sylla et al. [19], important aspects of climate change over West Africa will be reduction of the length of the rainy season and the growing season, increase in the intensity of extremes before mature monsoon season, and an extension of torrid, arid and semi-arid conditions. In such forecasted conditions it is important to assess the resistance/resilience to stress conditions of the crops and cultivars presently grown as well as the performance of viable alternatives. One important issue in the adaptation and mitigation of climate change is the search for agriculture crops and cultivars that can grow in the future climatic conditions [20].

Numerous studies have shown that water deficiency is one of the most important factors affecting plant growth [21–25]. In the case of maize, Heisey and Edmeades estimated 19 years ago, that drought stress was affecting 20% to 25% of the planting areas around the world [26]. More recently, other researchers [27,28] suggested that the severity and frequency of drought will probably increase in a near future.

Multiple effects of water shortage in plant cells, such as reduction of leaf water content, PSII photochemical efficiency, and photosynthetic pigments, have been reported in species as diverse as *Eragrostis curvula* (weeping lovegrass) [29], *Zea mays* (maize) [30], *Coffea canephora* (robusta coffee) [31], and *Solanum tuberosum* (potato) [32]. However, responses such as osmotic adjustment, antioxidant defense (increase of antioxidant enzymes' activities

and high content of non-enzymatic components such as ascorbic acid), and the activation of regulatory and functional genes involved in the perception and transmission of drought stress signal [22,31,33,34] have been described as useful mechanisms by which plants attenuate the damage caused by drought. In fact, dehydration is also reversible and full recovery is possible, but all depends on the severity of stress, the plant age, and the capacity to deal with the water deficit [35–37]. Particularly in areas experiencing significant environmental constraints, as is the case of drought stress, plant landraces are rightfully known as the backbone of sustainable food production [7]. However, protecting landraces from genetic erosion and valuing their potential for plant breeding and sustainable food production requires in-depth understanding of their phenotypic traits [9]. It is therefore important to know the limits of resilience of different maize landraces to drought stress. Hence, in this work, we evaluate the resistance and resilience to drought stress of four maize genotypes: two landraces currently grown in Guinea-Bissau and two elite cultivars.

2. Materials and Methods

2.1. Plant Material and Experimental Setup

Four different maize genotypes were used: two landraces from Guinea-Bissau, which were named as GA and GV following the color of the seeds [GA, yellow (in Portuguese, *amarelas*) seeds; GV, red (in Portuguese, *vermelhas*) seeds], and two commercial genotypes, P9838 and P0023 (both with tolerance to drought of 7 on a scale of 10) from DuPont Pioneer. The variety P0023 is an Optimum AQUAmax® hybrid, with improved water assimilation and usage (Accelerated Yield Technology (AYTTM) system); thus, it is expected to present a higher degree of tolerance to water deficit conditions [38]. Guinea-Bissau seeds were purchased at the Bandim market in Bissau and are from landraces commonly used by small farmers in rainfed cropping.

Before the germination process, the seeds were sterilized with 10% (*v*/*v*) commercial bleach, washed with demineralized water, and left in ultrapure water overnight. The next day, 20 seeds of each variety were placed on wet papers in trays sterilized with ethanol 70%, covered with transparent film, and left in a dark chamber at room temperature until radicle emergence. After 3 days, 10 seedlings of each genotype were transferred to 1 L pots (1 per pot) with soil supplemented with a surface fertilization (NPK 16-10-10 (nitrogen 16%–phosphoric anhydride 10%–potassium oxide 10%) + Mg-S 2-12 (magnesium oxide 2%–Sulfuric acid 12%); Flortis, Orvital S.p.A., Italy).

Pots were placed in controlled environment at a walk-in phytotron (Fitoclima 5000 EH, Aralab, Rio de Mouro, Portugal) with the following conditions: relative air humidity 50%, photosynthetically active radiation 200 µmol/m^{2}s, photoperiod 16 h, and temperature 25/18 °C (day/night). The plants were watered whenever necessary. Then, 18 days after the beginning of germination, the first measurements and sampling were done (control situation, C). Watering was withheld 23 days after germination, and 7 days later (day 30), a first set of measurements and sampling was done in water-stressed plants (S1), followed by a second set of measurements and sampling (S2) 5 days later (day 35 after germination). After completing these measurements and sampling, plants were immediately rewatered (R) and their recovery was assessed by measurements and sampling performed 6 days later (day 41 after germination).

2.2. Determination of Leaf Relative Water Content (RWC)

Leaf samples were weighed to obtain the fresh weight (FW). They were then left completely immersed in water overnight and in the next day weighed again to obtain the turgid weight (TW). Lastly, the leaf segments were left at approximately 70 °C until they were completely dry for the determination of the dry weight (DW). The leaf relative water content was calculated according to Catsky [39].

2.3. Chlorophyll Fluorescence Analysis

Chl. fluorescence was measured in vivo at each of the four conditions (C, S1, S2, and R), using a plant efficiency analyzer (Handy PEA, Hansatech Instruments, King's Lynn, UK). Samples were adapted to darkness for 10 min by placing light-withholding leaf clips and then exposed to a saturating light pulse with sufficient intensity to ensure closure of all photosystem II reaction centers (PSII RC) (3500 μmol/m^2 s). The data obtained were used for calculations according to the JIP test equations [40,41]. Four fluorescence intensity values from original measurements were used: the minimal fluorescence at 50 μs, when all PSII RCs were open (F_o); the fluorescence emission at 300 μs ($F_{300\mu s}$); the maximal fluorescence emission when all PSII RCs were closed (Fm); and the fluorescence intensity at 2 ms, the J step (Fj). From these four points, two parameters were directly obtained [42]: Vj, the relative variable fluorescence at 2 ms, and Mo, the initial slope, defined as the net ratio of RC closure. Based on these parameters, the PEA software automatically computed the performance index (PI), the maximum quantum efficiency of PSII (Fv/Fm), and the specific energy fluxes per RC: absorbed (ABS/RC), trapped (TR_o/RC), and dissipated as heat (DI_o/RC).

2.4. Photosynthetic Pigments Determination

Leaf samples for photosynthetic pigments determination were obtained immediately after fluorescence analysis through excision of part of the tip of the leaves Pigments were extracted through the addition of 1 mL methanol 100% to 0.9 cm diameter discs samples in centrifuge tubes. The samples were kept in the dark at −20 °C in the freezer for 72 h and then centrifuged at 14,000× *g* for 3 min. Supernatants were immediately subjected to spectrophotometric readings (Spectronic Helios β, Thermo Electron Corporation, Hemel Hempstead, UK) at wavelengths of 471, 652, and 665 nm to determine chlorophyll and carotenoid concentrations. Pigments were quantified according to equations of Lichtenthaler [43]. Results were expressed in milligram of pigments per gram of leaf dry weight.

2.5. Fatty Acids Profiling

The fatty acids analysis was performed as previously described [44,45]. Fatty acid methyl esters were prepared by incubation of 40 mg of leaves in freshly prepared methanol-sulfuric acid (97.5:2.5, *v*/*v*) at 70 °C for 60 min. FAMEs were recovered using 3 mL of petroleum ether and 2 mL of ultrapure water, followed by a centrifugation per 5 min. The upper phase was dried under a N_2 flow and resuspended in 40 μl of hexane. One microliter of the FAME solution was analyzed by gas chromatography (GC) (Varian 430-GC gas chromatograph, Varian Inc., Palo Alto, California, U.S.). Heptadecanoate (C17:0) was used as an internal standard.

Fatty acids were identified by comparison of their retention times with standards (Sigma-Aldrich) and chromatograms were analyzed using the Galaxy software. The double bond index (DBI) was calculated as described in Feijão et al. [46]:

$$DBI = 2 \times [(\%\ monoenes) + (2 \times \%\ dienes) + (3 \times \%\ trienes)]/100$$

2.6. RNA Extraction and cDNA Synthesis

Leaf samples were frozen in liquid nitrogen and grinded with a mortar and a pestle. RNA was extracted with the Spectrum™ plant total RNA kit (Sigma-Aldrich, St. Louis, MO, USA) following the supplier's instruction. Genomic DNA (gDNA) was digested using the on-column Dnase I digestion set (Sigma-Aldrich, St. Louis, MO, USA) following the supplier's instruction. RNA integrity was verified by agarose gel electrophoresis, and purity and concentration were accessed with Nanodrop equipment (ND-1000 spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). Prior to complementary DNA (cDNA) synthesis, all samples were analyzed for genomic DNA contamination through a quantitative real-time polymerase chain reaction (qPCR) of the reference gene *Elongation Factor 1-alpha* (*EF1α*) [45]. Complementary DNA was synthesized from 2.5 μg of total RNA

using RevertAid®H minus reverse transcriptase (Fermentas, ON, Canada) anchored with Oligo (dT)23 primer (Fermentas, ON, Canada) as described in Nascimento et al. [47].

2.7. Real-Time PCR (qPCR)

The qPCR experiments were performed in a StepOne™ real-time PCR system (Applied Biosystems, Sourceforge, Foster City, CA, USA) using the Maxima™ SYBR green qPCR master mix (2×) kit (Fermentas, Burlington, ON, Canada) following the manufacturer's instructions. Each set of reactions included a control without a cDNA template. Maize Phospholipase D gene (D73410.1) was the target gene and *Elongation Factor 1-alpha (EF1α)* and *Tubulin beta-4 chain (TUB4)* were used for data normalization. These reference genes were chosen because of their stability in maize plants suffering from drought stress [45]. Thermal cycling started with a 95 °C denaturation step for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s and annealing for 30 s. Three biological replicates and two technical replicates were used for each sample. Gene expression (fold change) was calculated according to the Hellemans' method [48].

2.8. Statistical Analysis

Owing to the absence of normality and homogeneity of variances of the obtained data, the results were analyzed through the non-parametric Kruskal–Wallis (KW) test using IBM SPSS Statistics 27 software. Given the fact that the KW test assesses only one dependent variable, two separate tests were performed. The first test assessed if the distribution of the data for each genotype (P0023, P9838, GA, GV) behaved similarly in each dependent variable, therefore highlighting differences between genotypes. The second test assessed if the data behaved similarly depending on the growth conditions (C, S1, S2, R) within each genotype in each dependent variable, therefore highlighting differences due to growth conditions. Statistical significance was considered when $p < 0.05$.

3. Results

No major differences were found between genotypes in the response of relative water content to water stress. All genotypes in the control and stress 1 groups had the RWC above 75% (Figure 1).

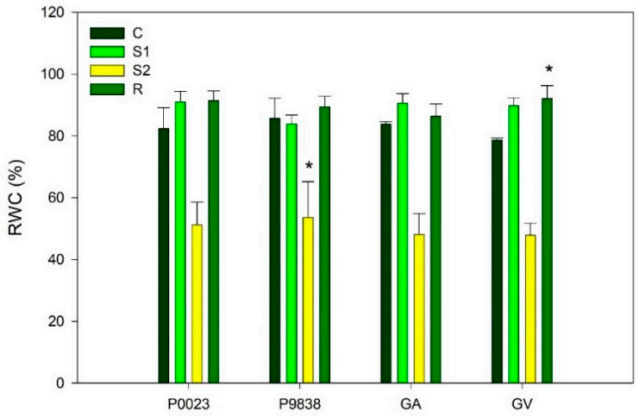

Figure 1. Leaf relative water content of all studied genotypes in the different conditions (mean ± standard deviation; $n = 10$). Well-watered plants (C); plants stressed for 7 (S1) and 12 (S2) days; rewatered plants (R). Asterisks indicate significant differences between drought conditions and control group ($p \leq 0.05$).

The RWC percentage decreased substantially by almost half in the stress 2 group. However, after rewatering, plants were able to recover their initial RWC. In fact, in all genotypes, the RWC values at stress 2 were significantly lower than at stress 1, as well as in the rewatered group ($p < 0.05$) (Figure 1).

In control conditions (C), significant differences on photochemical parameters between genotypes were observed (Figure 2).

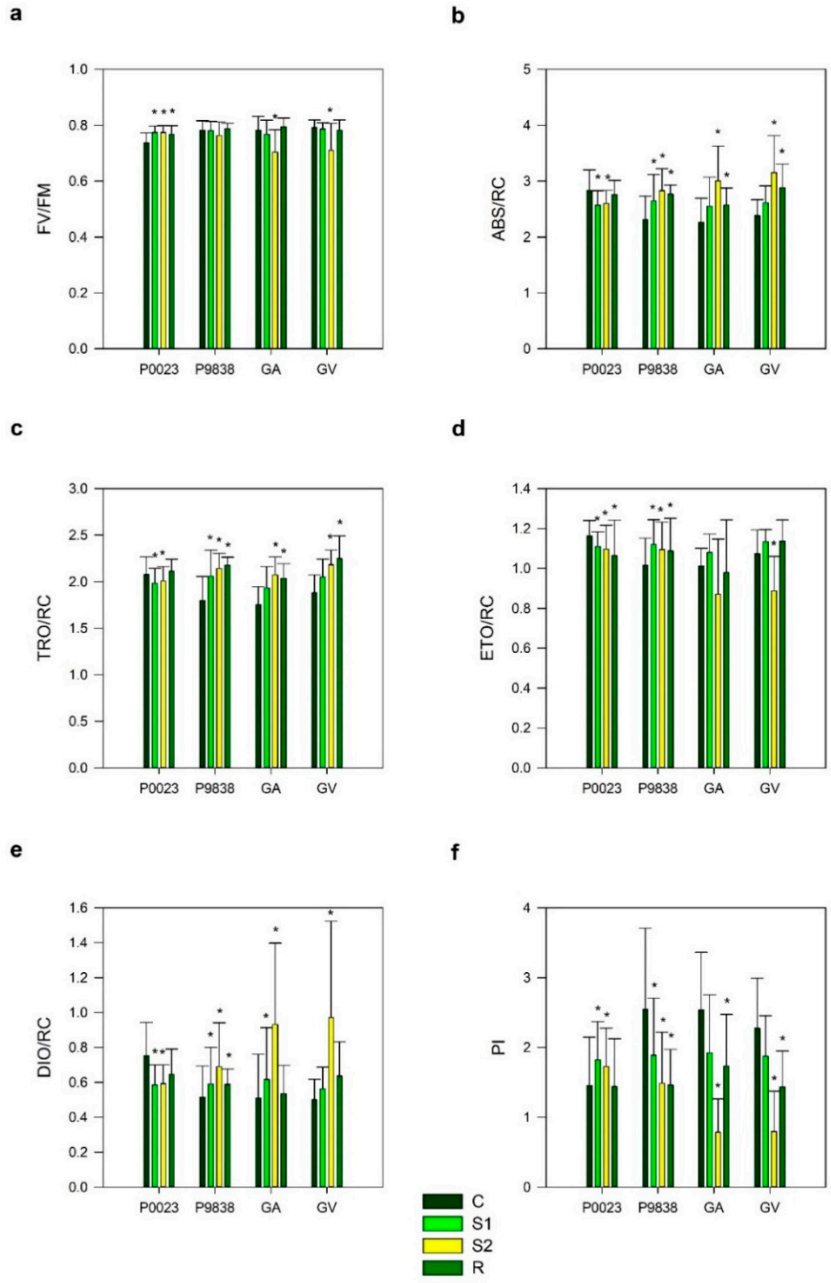

Figure 2. Chlorophyll fluorescence of maize plants subjected to drought stress and rewatered. (**a**) Maximum quantum efficiency of PSII (Fv/Fm); (**b**) performance index (PI); (**c**) electron transport energy per RC (Eto/RC); (**d**) trapped energy flux per RC (TRo/RC); (**e**) absorbed energy flux per RC (ABS/RC); (**f**) energy dissipation as heat per RC (DIo/RC). Well-watered plants (C); plants stressed for 7 (S1) and 12 (S2) days; rewatered plants (R). Values correspond to mean ± standard deviation (n = 10); asterisks indicate significant differences between drought conditions and control group ($p \leq 0.05$).

In control plants (C) P0023 presented a lower Fv/Fm and PI and a higher TRO/RC and DIO/RC than the other genotypes. However, during the initial phase of stress (S1), photochemical parameters converged and significant differences between genotypes were almost absent (Figure 2). With the progress of stress conditions (S2) differences between the genotypes reappeared. GA and GV presented a lower Fv/Fm, PI, and ETO/RC than

the commercial genotypes. Except for FV/Fm on P9838 and Eto/RC in GA, all fluorescence parameters departed significantly from the control value in S1 and/or S2 ($p < 0.05$).

The Guinea-Bissau genotypes presented a sharper decrease of leaf chlorophyll *a* and total chlorophyll content under stress conditions, and no recovery after rewatering, presenting significantly lower values at S1, S2, and R in relation to the control ($p < 0.05$) (Figure 3). Except for a significant decrease for Chl. *a* in rewatered P9838, significant changes of the Chl. *a* and total Chl. content were not observed in the different conditions on the elite cultivars.

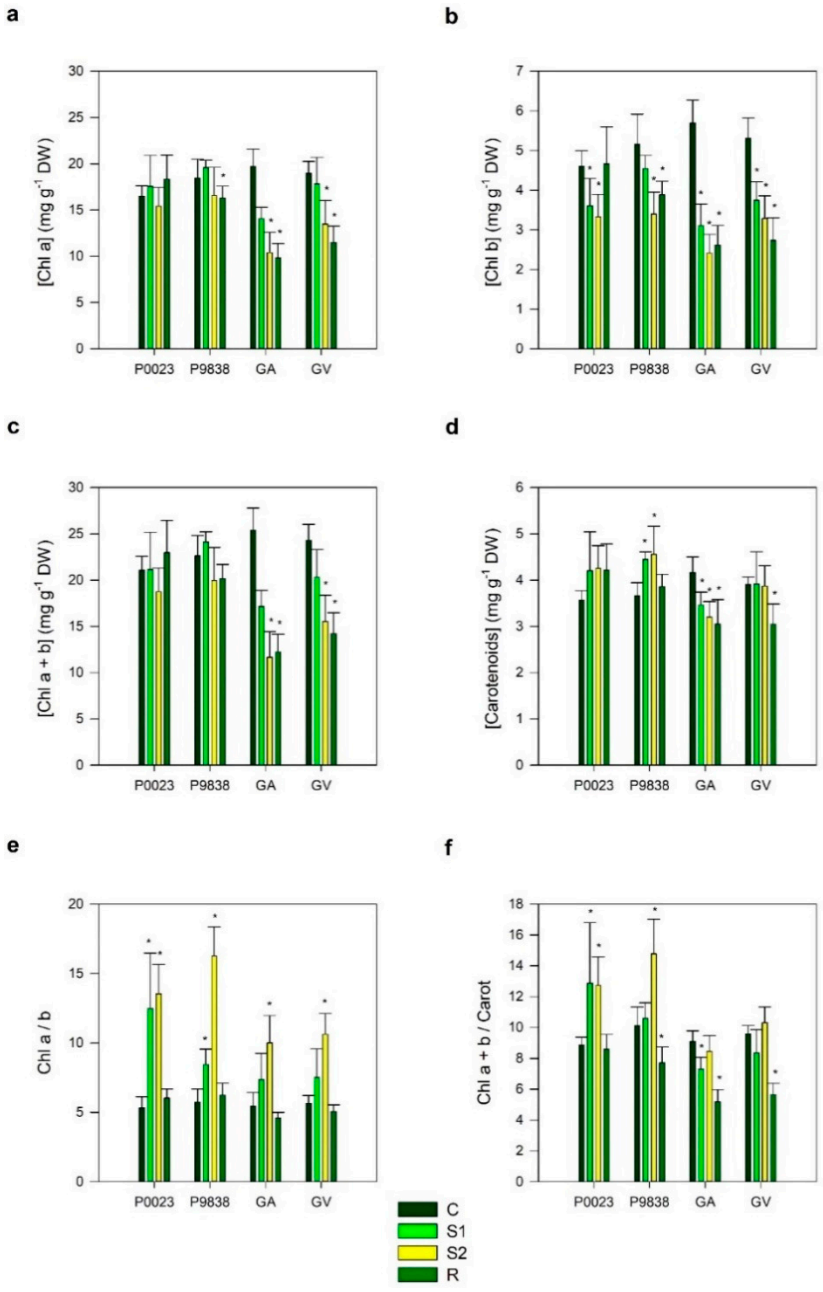

Figure 3. Photosynthetic pigments concentration of maize plants subjected to drought stress and rewatered. (**a**) Chlorophyll *a* (Chl a); (**b**) chlorophyll *b* (Chl b); (**c**) total chlorophyll (Chl a + Chl b); (**d**) total carotenoids (carotenoids); (**e**) ratio chlorophyll *a*/chlorophyll *b* (Chl a/Chl b); (**f**) ratio total chlorophyll/total carotenoids (Chl a + b/Carot). Well-watered plants (C); plants stressed for 7 (S1) and 12 (S2) days; rewatered plants (R). Values correspond to mean ± standard deviation ($n = 10$); asterisks indicate significant differences between drought conditions and control group ($p \leq 0.05$).

A similar, albeit more complicated trend was observed in total carotenoids (Figure 3d). In fact, no changes in total carotenoids content were observed in P0023, but in P9838, an increase was even observed in S1 and S2 in relation to C, albeit at R the value decreased to near the control ($p < 0.05$). Between the Guinea-Bissau lines some differences were also recorded: whereas in GA a decrease in total carotenoids content was observed in all treatments in relation to C, in GV, the carotenoids content was only lower at R than at C. In the Pioneer genotypes, the ratio Chl. a/b increased in relation to C at S1 and at S2, but such an increase was observed on the Guinea-Bissau genotypes only at S2 ($p < 0.05$) (Figure 3e). In addition, the ratio Chl. a+b/carot increased with stress in relation to C in the Pioneer genotypes (except in S1 for P9838), but was unchanged in the African genotypes (except for GA at S1) ($p < 0.05$) (Figure 3f).

The most abundant fatty acid present in maize leaves is α-linolenic acid (C18:3), which accounts for ~70% of all fatty acids. By order of abundance the following fatty acids were detected: the saturated palmitic acid (C16:0) and the unsaturated linoleic acid (C18:2). The saturated myristic (C14:0) and stearic (C18:0) acids as well as the plastidial-specific trans-hexadecanoic acid (16:1*t*) were present in lower amounts and only residual amounts of oleic acid (C18:1) were detected (Figure 4).

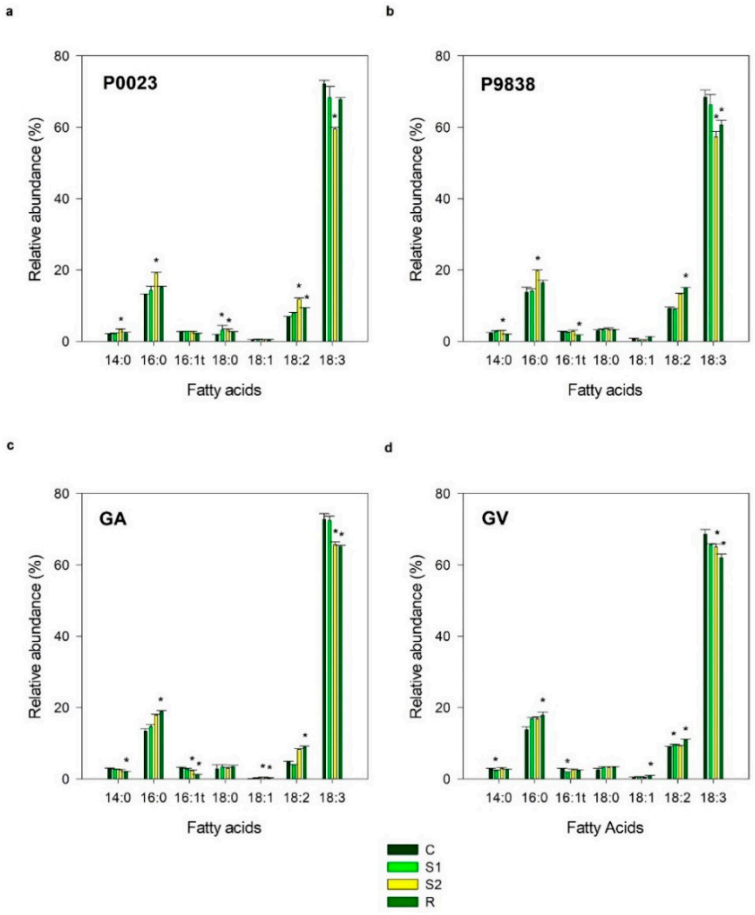

Figure 4. Fatty acids profiles of maize plants subjected to drought stress and rewatered. (**a**) P0023; (**b**) P9838; (**c**) GA; (**d**) GV. Myristic acid (C14:0), palmitic acid (C16:0), trans-hexadecanoic acid (16:1*t*), stearic acid (C18:0), oleic acid (C18:1); linoleic acid (C18:2), and α-linolenic acid (C18:3). Well-watered plants (C); plants stressed for 7 (S1) and 12 (S2) days; rewatered plants (R). Values correspond to mean ± standard deviation (n = 3-4); asterisks indicate significant differences between drought conditions and control group ($p \leq 0.05$).

Regarding the total fatty acid content, this parameter was not affected at the early stages of drought (S1), and even suffered an increase for P9838 and GV, whereas a general

tendency to decrease at S2 was observed in all genotypes. Although rewatered plants tended to recover the initial lipid contents, this was not the case for GA (Figure 5d).

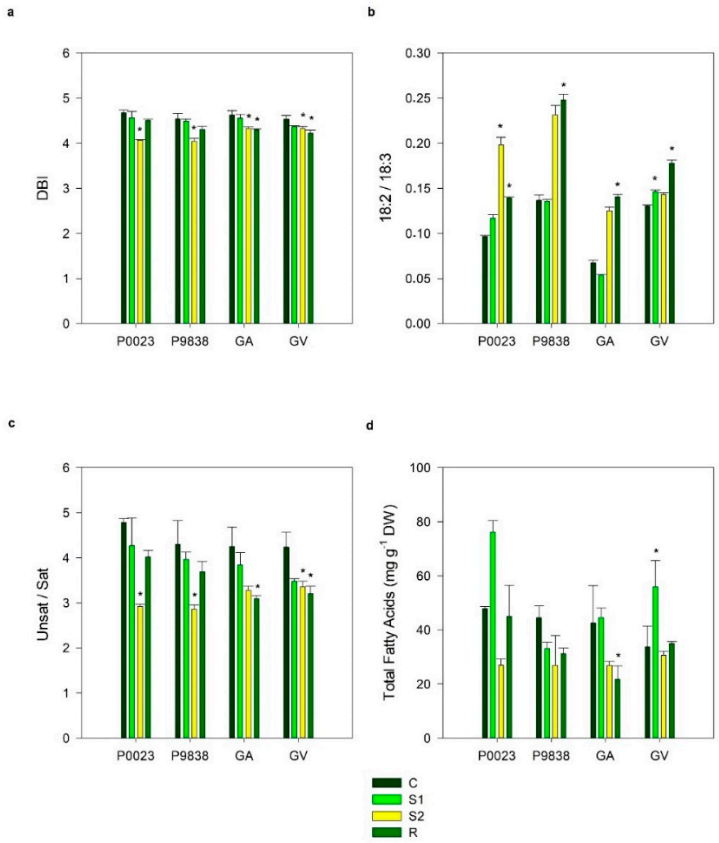

Figure 5. The double bond index (DBI) (**a**), ratio linoleic-to-α-linolenic acid (C18:2/C18:3) (**b**), ratio unsaturated-to-saturated fatty acids (Unsat/Sat), (**c**) and total fatty acids content (**d**) of leaf samples of maize plants subjected to drought stress and rewatered. Well-watered plants (C); plants stressed for 7 (S1) and 12 (S2) days; rewatered plants (R). Values correspond to mean ± standard deviation, n = 3–4. Asterisks indicate significant differences between drought conditions and control group ($p \leq 0.05$).

In the four genotypes studied, the drought treatment S2 resulted in a reduction in C18:3 relative amounts and the opposite occurred for C16:0 (Figure 5a–d). Rewatering reversed this effect in P0023 and partially in P9838 (only for C16:0), but not for the other two genotypes. The reduction of C18:3 was accompanied by an increase in its biosynthetic precursor, C18:2, for all the genotypes after rewatering, for GV at S1, and for P0023 at S2. These drought-induced alterations in the relative amounts of the two polyunsaturated FAs resulted in a general trend for an increase in the C18:2/C18:3 ratios (Figure 5b). The levels of the saturated C18:0 were also higher in the S1 and S2 samples of P0023, as well as the R leaves of P9838. S2 also caused increases of C14:0 S2 in P0023, P9838, and GV, whereas an increase during recovery was observed in GA. Concerning the C16:1*t* amount, an early reduction (S1) was seen in GV, and in GA, this FA was less abundant in S2 and R. Although in P9838 the C16:1*t* content was lower during R, no changes were observed in P0023 in any of the samples analyzed. All these changes in the FA profiles resulted in lower double bond indexes (DBI), and lower ratio of unsaturated to saturated (Unsat/Sat) for all the genotypes at S2, and only P0023 and P9838 were able to present values similar to controls after rewatering (Figure 5a,c).

Drought stress also modulated PLD gene expression in the four genotypes. During S1, PLD expression was significantly augmented in P9838 and GA, while in both P0023 and GV, there was a downregulation of this gene (Figure 6).

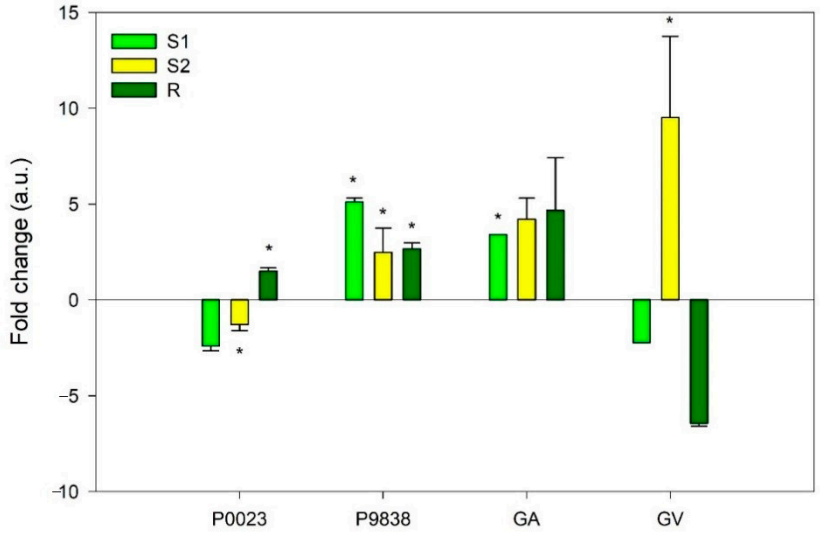

Figure 6. Phospholipase D gene expression in the four genotypes under study; plants stressed for 7 (S1) and 12 (S2) days; rewatered plants (R). Values correspond to mean ± standard deviation, $n = 6$. Asterisks indicate significant differences between drought conditions and control group ($p \leq 0.05$).

In S2, PLD remained downregulated in P0023, while in the other three genotypes it was upregulated. During the recovery period, P0023, P9838, and GA presented an upregulation of PLD, while in GV, this gene was negatively modulated (Figure 6).

4. Discussion

Like any living being, much of the mass of plants are made up of water, which is therefore a limiting factor for their growth. All the studied genotypes presented similar values of RWC among them in each of the conditions studied (C, S1, S2, and R). A similar RWC was also observed among Mozambique maize genotypes under control, drought-stressed, and rewatered plants [49]. A soil water "deficit induced" alterations on plants' normal water content, which decreased with prolonged drought stress, as can be confirmed by our RWC results at S2 (Figure 1) and as have been commonly observed in drought stress and recovery studies [50]. Nevertheless, the shorter water stress period (S1), albeit not affecting RWC, impacted several physiological and metabolic processes, as shown by several Chl. *a* fluorescence parameters, which presented a significant departure from the control values at S1, particularly in the Pioneer genotypes (Figure 2). This indicates that the plants, despite being under a very mild drought stress at S1, which did not decrease leaf cellular water content, were already sensing the decrease of soil water content and changing their metabolism more notably in the Pioneer genotypes. The increase of RWC observed after rewatering, reaching the control levels in all genotypes except GV, was also found in several experiments related to the effects of drought in maize [30,50,51]. According to Franks and Farquhar [52], an impairment of the RWC recovery after rewatering may occur when water stress causes cellular damage or loss of stomatal regulation. Since such impairment was not detected in our experiments, severe cellular damage and loss of stomatal function may be excluded. In general, as referred previously, the photochemical activity was already moderately affected in the initial phase of water stress (S1), as revealed by the few alterations, mostly in the Pioneer genotypes, found on the Chl. *a* fluorescence parameters (Figure 2). Since photosystems are involved in the capture of the light that triggers the process of photosynthesis, their damage causes a decrease in the photosynthetic rate. However, changes in the function of the photosystems may also have a protective role, as might be the case of the alterations found at S1. In fact, in a study using Arabidopsis, Harb et al. [53] revealed that under moderate drought, the photosynthesis rate remained normal, and Lima et al. and Marques da Silva and Arrabaça [31,54] revealed that upon moderate water stress, photosynthesis decreases mainly because of stomatal closure. Given

these observations, it can be suggested that the effects of S1 were not sufficient to cause damages in PSII. It is interesting to note that the Pioneer genotypes showed many more changes in the fluorescence parameters at S1 than the Guinea's genotypes, suggesting that the former may be able to sense the decrease of soil water content in anticipation and adjust the photochemical activity accordingly. In the case of the longer and more intense water stress (S2), differences in the Chl. *a* fluorescence parameters of control and stressed plants were accentuated. The maximum potential efficiency of PSII photochemistry (Fv/Fm) was strongly reduced in the GA and GV plants during S2 (Figure 2a). In contrast, no changes were observed in P9838 and a significant increase ($p < 0.05$) was even found at S2 in P0023. The values of this parameter were reported to be very stable among different species, averaging 0.83 [55] in unstressed plants and, despite the decline observed in the varieties GA, GV, and P0023, Fv/Fm still presented relatively high values, circa 0.7, confirming the idea that this parameter is very resistant to drought stress conditions [54]. A sustained decrease in Fv/Fm indicates the occurrence of photoinhibitory damage [56]; therefore, it is possible that the Guinea's plants suffered damage in the photosynthetic apparatus, which notoriously contrasted with the findings in the Pioneer genotypes. The performance index (PI) significantly decreased in GA, GV, and P9838 during S2, when compared with the control, contrasting with the increase observed in P0023 ($p < 0.05$) (Figure 2b). PI is a good indicator of plant vitality, expressing their ability to avoid drought and maintain physiological activity at a certain level during stress [57], and unlike Fv/Fm, which is only dependent of the primary photochemical reactions at PSII, PI is a very sensitive indicator of the integral photophysiological status of plants, from the efficiency of light energy capture to the function of the Calvin–Benson cycle via the electron transport rate. Therefore, the decrease in PI values may be interpreted as evidence of the plants failure to maintain the normal PSII function [15]. We must emphasize, however, that P0023, which showed an increase in PI at S1 and S2, presented a much lower value at C ($p < 0.05$) when compared with the C values of the other genotypes, suggesting a lower photochemical performance under optimal irrigation conditions. The specific energy fluxes of absorption, trapping, and dissipation per RC (ABS/RC, TRo/RC, and Dlo/RC) were higher under drought stress (Figure 2d–f) for P9838, GA, and GV, indicating that the energy absorbed (ABS/RC) and trapped (TRo/RC) by active RC increased under water stress, owing to the inactivation of a fraction of RCs, and the dissipation as heat (Dlo/RC) increased because of accumulation of inactive RCs [15]. In contrast, a decrease of these parameters with water stress was found in P0023, suggesting the absence of inactivation of RCs. However, P0023 and P9838 showed opposing trends in the response of ETo/RC to water stress (Figure 2c), with the electron transport rate per reaction center decreasing in the former and increasing in the latter ($p < 0.05$), suggesting that the resilience to oxidative stress (absence of RC inactivation) comes together with a lower photochemical activity. Furthermore, in wheat, Correia et al. [58] found a strong correlation between these energy fluxes and the antioxidant capacity of leaves under drought stress. Since drought stress causes the loss of photosynthetic reaction centers, the loss of chlorophyll *a* content detected in all except P0023 plants during S2 was expected. In addition, GV decreased Eto/RC under drought stress, but no changes ($p > 0.05$) were observed in GA.

The chlorophyll *a*, *b*, and *a* + *b* content decreased under drought in leaves of Guinea plants (Figure 3a–c) showing greater reduction during S2. In contrast, the concentration of these pigments in Pioneer plants did not change significantly in stress conditions when compared with the control ($p > 0.05$) (Figure 3a–c), being particularly stable in P0023. Chandrasekar et al. [59] reported that drought-tolerant genotypes were able to maintain higher chlorophyll content than susceptible genotypes, suggesting that the Pioneer genotypes, most notably P0023, were less susceptible to drought than the Guinea's ones, which is in line with the results of Chl. *a* fluorescence. Nevertheless, the decrease in chlorophyll content in GA and GV during drought stress may be a defense mechanism against photooxidative damage, which occurs when photosynthesis is inhibited and light-exciting energy is in excess [60,61]. In fact, the significant increase of the Chl. *a*/Chl. *b* ratio in

stressed plants of all genotypes (Figure 3e) shows that the decrease on chlorophyll content occurs mostly at the photosynthetic antenna (where Chl. *b* is present), and much less at the reaction center (where only Chl. *a* is present), suggesting a mechanism to lower the energy input at the RC and electron transport chain, as previously seen in acclimation to high light [62]. In fact, chlorophyll occurs in a ratio (a/b) of approximately 3 to 1 [43], but growth conditions and environmental factors can modify this chlorophyll a/b ratio. The increase of carotenoid content also suggests increased photoprotection, but it was only found in P9838; in GA, carotenoid content decreased in stressed plants ($p < 0.05$) and no significant change was observed in P0023 and GV (Figure 3d). Therefore, the ratio between Chl. $a + b$ and carotenoids also did not present a clear pattern of change: it increased at P0023 and P9838 (but only at S2 in the latter) and decreased at GA, whereas no significant changes were observed at GV (Figure 3f).

The oxidative stress can also be related to fatty acids composition because it can result in membrane lipid peroxidation [63], and consequently, the decrease in total fatty acids amount. The fatty acid (FA) content and composition of leaf lipids were also affected under water stress. A general trend for a reduction in the total FA amounts was observed for all the genotypes. However, in P0023 and GV, this value suffered an increase at S1 and returned to control levels after rewatering. Under severe stress the inhibition of lipid biosynthesis and increase of oxidative stress damage as well as lipolytic activities are known to contribute to the reduction of lipid content [64], which was previously described for other plants [65–68]. The thylakoid membrane contains a high percentage of galactolipids [69], which are mainly build of C18:3. Moreover, the only phospholipid present in thylakoids is phosphatidylglycerol, characterized by the presence of C16:1*t* [65]. The high content of polyunsaturated FAs, such as C18:3 in membrane lipids, is a crucial factor for membrane fluidity [70]. However, membrane restructuring with lower amounts of polyunsaturated FAs is considered an adaptation to osmotically stressful environments. Our results show that water stress caused a reduction in the C18:3 contents of maize leaves. Among the four genotypes, only P0023 was able to increase its C18:3 levels after rehydration. In parallel to the C18:3 decrease, an increase of its precursor, C18:2, was observed in drought-stressed samples, which can reflect a decrease in galactolipid contents [65] and/or a reduction in the desaturase activity, converting C18:2 in C18:3. Increases in the C18:2/C18:3 ratio have been previously observed in response to water deficit, and considered a mechanism of adaptation to water deficit [71]. Although increases in the C18:2/C18:3 ratios were observed in the four genotypes studied, well-watered GA plants had the lower basal level, and in both Pioneer cultivars, the higher drought-induced increases were observed. Moreover, only in P0023 did this ratio decrease again after rewatering, while it remained high in the three remaining genotypes under study. DBI values and Unsat/Sat ratios are used as measure of membrane fluidity [72,73]. Our results show that a prolonged stress decreased both DBI and Unsat/Sat values in all the genotypes; however, only in Pioneer plants, rewatering resulted in a return to values found in well-watered plants. In addition, considering the relative abundance of the plastidial-specific FA C16:1*t*, reductions were seen for both the Guinea-Bissau landraces under water deficit, whereas no changes were observed for P0023. The overall decreases in the abundance of C18:3 and C16:1*t* are likely to be associated to photosynthesis impairment under water deficit observed in maize plants [74].

Taken together, our results on the drought-induced changes in the fatty acid contents and composition agree with the higher photosynthetic performance displayed by Pioneer genotypes under drought conditions, especially P0023, and a higher damage of the photosynthetic apparatus suggested for Guinea-Bissau plants in response to this stress, which also appear to be less able to recover after rewatering.

Phospholipase D (PLD) is an enzyme that hydrolyses phosphatidylcholine into phosphatidic acid and choline, and it has been reported by several investigators as playing an important role in plants under abiotic stress, being involved in signal transduction, cellular regulation, hormone signaling, and stress response [75–77]. In a water stress situation,

PLD is involved in stomatal closure in response to ABA increase [77,78]. For that reason, the increase in PLD expression during stress observed in most of the genotypes studied (Figure 5), essentially during S2, was expected. Nevertheless, Hayano-Kanashiro et al. [79] investigated the effect of 17 days of drought and recovery in maize and proved that PLD was induced under drought stress and repressed upon recovery (rewatering) for all three landraces studied, which did not happen with the Pioneer plants during recovery. The hypothesis to explain this divergence of results is to assume that this increase in the expression level of PLD during recovery was not significant or, in case of GA plants whose expression was higher, the recovery rate was slower.

5. Conclusions

Overall, P0023 presented the most distinctive response to drought stress, with P9838 showing an intermediary behavior between P0023 and the Guinea's landraces, where only minor differences between them were found. P0023 was the only genotype that almost returned to the control situation after rewatering (only 5 out of the 25 traits analyzed remained altered in relation to control, contrasting with 14, 18, and 15 in P9838, GA, and GV, respectively). This enhanced capacity of P0023 to recover from drought stress may be related with a more precocious sensing and response to stress. In fact, at S1, P0023 was the genotype that already presented more traits altered in relation to C (9/25), but at least some of them were linked to adaptative responses, as is the case of the significant increase on Fv/Fm, in sharp contrast with all other genotypes. The most remarkable aspect of the Guinea's genotypes, on the contrary, was a dramatic decrease of Chl. *b* (which also impacted the decrease of total Chl.), already present at S1, and still present at R. This decrease is only partly visible on the increase of the Chl. *a*/*b* ratio, which shows an adaptative response to adjust the photosynthetic antenna to the decreased energy demand under drought, and was much more notorious on the Pioneer genotypes, suggesting that the decrease of Chl. content on the Guinea's genotypes is a deleterious rather than adaptative process. This work helped to disentangle the distinct photophysiological, biochemical, and molecular mechanisms underlying the response of commercial and traditional maize genotypes to drought stress, paving the way for the selection of new breeding traits for increased drought resilience. Even though elite maize cultivars became major players in global production, maize landraces are still key actors on the food security of remote rural communities. However, there is a deep concern with the tolerance and adaptive capacity of landraces to the new climate change conditions. Alterations may be of such scale and speed that the evolutionary potential of crop populations may not suffice [80]. Breeding programs to develop landraces for abiotic stress resilience, therefore, are a strategy to assure the sustainability of local agricultural systems [8]. Such effort, however, requires the systematic characterization of the physiological, biochemical, and molecular responses to stress, i.e., the functional phenotyping of landraces in close relation to their genotyping. Finally, in addition to the importance of landraces for the sustainability of agricultural systems, their importance as a source of knowledge and as a cultural heritage is unquestionable [81], and every effort must be made to conserve them.

Author Contributions: Conceptualization, J.M.d.S., L.C., and A.F.; methodology, M.T., A.R.M., and A.F.; writing—original draft preparation, M.T.; investigation, M.T and E.F.; writing—review and editing, J.M.d.S., A.F., L.C., and A.R.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FCT/BioISI (UID/MULTI/04046/2019), FCT/cE3c (UIDB/00329/2020) and the FCT funded research project INTERPHENO (PTDC/ASP-PLA/28726/2017).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable

Acknowledgments: We are grateful for the technical support of Manuela Lucas. We are also grateful for the support of FAO Guinea-Bissau in the scope of the project "Appui à la lutte contre les maladies et les ravageurs d'anacardier (Anacardium occidentale) en Guinée-Bissau" (TCP_GBS_301). Thanks are also due to Colégio Tropical, Universidade de Lisboa, and to João Serôdio.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Silva, J.L. *O Zea Mays e a Expansão Portuguesa*; Instituto de Investigação Científica Tropical: Lisboa, Portugal, 1998; p. 212.
2. Campos, H.; Cooper, M.; Habben, J.E.; Edmeades, G.O.; Schussler, J.R. Improving drought tolerance in maize: A view from industry. *Field Crop. Res.* **2004**, *90*, 19–34. [CrossRef]
3. Kim, S.G.; Bae, H.H.; Jung, H.J.; Lee, J.-S.; Kim, J.-T.; Go, T.H.; Son, B.-Y.; Baek, S.-B.; Kwon, Y.-U.; Woo, M.-O.; et al. Physiological and protein profiling response to drought stress in KS141, a Korean maize inbred line. *J. Crop. Sci. Biotechnol.* **2014**, *17*, 273–280. [CrossRef]
4. Moola, S. *Africa's Green Revolution Drought Tolerant Maize Scam*; ACB Briefing Paper No. 12; African Centre for Biodiversity: Melville, South Africa, 2010; p. 18. Available online: https://www.acbio.org.za/wp-content/uploads/2015/02/ACB-Africa-Drought-Tolerant-Maize-2010.pdf (accessed on 1 February 2021).
5. Santpoort, R. The Drivers of Maize Area Expansion in Sub-Saharan Africa. How Policies to Boost Maize Production Overlook the Interests of Smallholder Farmers. *Land* **2020**, *9*, 68. [CrossRef]
6. Pinheiro de Carvalho, M.A.; Bebeli, P.J.; Bettencourt, E.; Costa, G.; Dias, S.; Santos, T.M.; Slaski, J.J. Cereal landraces genetic resources in worldwide GeneBanks. A review. *Agron. Sustain. Dev.* **2013**, *33*, 177–203. [CrossRef]
7. Kumar, A.; Verma, R.P.; Singh, A.; Sharma, H.K.; Devi, G. Barley landraces: Ecological heritage for edaphic stress adaptations and sustainable production. *Environ. Sustain. Indic.* **2020**, *6*, 100035. [CrossRef]
8. Koutsika-Sotiriou, M.; Tsivelikas, A.L.; Gogas, C.; Mylonas, I.G.; Avdikos, I.; Traka-Mavrona, E. Breeding methodology meets sustainable agriculture. *Int. J. Plant Breed. Genet.* **2013**, *7*, 1–20. [CrossRef]
9. Newton, A.C.; Akar, T.; Baresel, J.P.; Bebeli, P.J.; Bettencourt, E.; Bladenopoulos, K.V.; Czembor, J.H.; Fasoula, D.A.; Katsiotis, A.; Koutis, K.; et al. Cereal landraces for sustainable agriculture. A review. *Agron. Sustain. Dev.* **2010**, *30*, 237–269. [CrossRef]
10. Collins, N.C.; Tardieu, F.; Tuberosa, R. Quantitative Trait Loci and Crop Performance under Abiotic Stress: Where Do We Stand? *Plant Physiol.* **2008**, *147*, 469–486. [CrossRef] [PubMed]
11. Reynolds, M.P.; Ortiz, R. Adapting crops to climate change: A summary. In *Climate Change and Crop Production*; Reynolds, M.P., Ed.; CABI: Oxfordshire, UK, 2010; pp. 1–8. [CrossRef]
12. Mahajan, S.; Tuteja, N. Cold, salinity, and drought stress. *Arch Biochem. Biophys.* **2005**, *444*, 139–158. [CrossRef] [PubMed]
13. Song, H.; Li, Y.; Zhou, L.; Xu, Z.; Zhou, G. Maize leaf functional responses to drought episode and rewatering. *Agr. Forest Meteorol.* **2018**, *249*, 57–70. [CrossRef]
14. Paviot, M.C.; Bresnyan, E.W., Jr.; Diaz, A. *Guinea Bissau: Unlocking Diversification to Unleash Agriculture Growth*; World Bank: Washington, DC, USA, 2019; p. 142. Available online: http://documents1.worldbank.org/curated/en/341991563831364596/pdf/Guinea-Bissau-ASA-Agriculture-sector-Report.pdf (accessed on 1 February 2021).
15. Fghire, R.; Anaya, F.; Ali, O.I.; Benlhabib, O.; Ragab, R.; Wahbi, S. Physiological and photosynthetic response of quinoa to drought stress. *Chil. J. Agr. Res.* **2015**, *75*, 174–183. [CrossRef]
16. Opitz, N.; Paschold, A.; Marcon, C.; Malik, W.A.; Lanz, C.; Piepho, H.P.; Hochholdinger, F. Transcriptomic complexity in young maize primary roots in response to low water potentials. *BMC Genom.* **2014**, *15*, 1–13. [CrossRef] [PubMed]
17. Challinor, A.; Wheeler, T.; Garforth, C.; Craufurd, P.; Kassm, A. Assessing the vulnerability of food crop systems in Africa to climate change. *Clim. Chang.* **2007**, *83*, 381–399. [CrossRef]
18. Tesfaye, K.; Gbegbelegbe, S.; Cairns, J.E.; Shiferaw, B.; Prasanna, B.M.; Sonder, K.; Boote, K.; Makumbi, D.; Robertson, R. Maize systems under climate change in sub-Saharan Africa: Potential impacts on production and food security. *Int. J. Clim. Chang. Str.* **2016**, *7*, 247–271. [CrossRef]
19. Sylla, M.B.; Nikiema, P.M.; Gibba, P.; Kebe, I.; Klutse, N.A.B. Climate Change over West Africa: Recent Trends and Future Projections. In *Adaptation to Climate Change and Variability in Rural West Africa*; Yaro, J., Hesselberg, J., Eds.; Springer: Cham, Switzerland, 2016. [CrossRef]
20. FAO. *Climate-Smart Agriculture Sourcebook*; FAO: Rome, Italy, 2017; p. 56. Available online: http://www.fao.org/3/i7994e/i7994e.pdf (accessed on 1 February 2021).
21. Efeoğlu, B.; Ekmekçi, Y.; Çiçek, N. Physiological responses of three maize cultivars to drought stress and recovery. *S. Afr. J. Bot.* **2009**, *75*, 34–42. [CrossRef]
22. Li, J.; Cang, Z.; Jiao, F.; Bai, X.; Zhang, D.; Zhai, R. Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage. *J. Saudi Soc. Agric. Sci.* **2017**, *16*, 82–88. [CrossRef]
23. Toumi, I.; Gargouri, M.; Nouairi, I.; Moschou, P.N.; Ben Salem-Fnayou, A.; Mliki, A.; Zarrouk, M.; Ghorbel, A. Water stress induced changes in the leaf lipid composition of four grapevine genotypes with different drought tolerance. *Biologia. Plantarum.* **2008**, *52*, 161–164. [CrossRef]
24. Wang, W.; Vinocur, B.; Altman, A. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta* **2003**, *218*, 1–14. [CrossRef] [PubMed]

25. Yang, S.; Vanderbeld, B.; Wan, J.; Huang, Y. Narrowing down the targets: Towards successful genetic engineering of drought-tolerant crops. *Mol. Plant* **2010**, *3*, 469–490. [CrossRef]
26. Heisey, P.W.; Edmeades, G.O. *Maize Production in Drought-Stressed Environments: Technical Options and Research Resource Allocation*; Part 1 of CIMMYT 1997/98 World Maize Facts and Trends; CIMMYT: Mexico City, Mexico, 1999; pp. 1–74. Available online: http://hdl.handle.net/10883/759 (accessed on 1 February 2021).
27. Dai, A. Increasing drought under global warming in observations and models. *Nat. Clim. Chang.* **2013**, *3*, 52–58. [CrossRef]
28. Mir, R.R.; Zaman-Allah, M.; Sreenivasulu, N.; Trethowan, R.; Varshney, R.K. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theor. Appl. Genet.* **2012**, *125*, 625–645. [CrossRef]
29. Colom, M.R.; Vazzana, C. Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping lovegrass plants. *Environ. Exp. Bot.* **2003**, *49*, 135–144. [CrossRef]
30. Fleta-Soriano, E.; Díaz, L.; Bonet, E.; Munné-Bosch, S. Melatonin may exert a protective role against drought stress in maize. *J. Agron. Crop Sci.* **2017**, *203*, 286–294. [CrossRef]
31. Lima, A.L.S.; DaMatta, F.M.; Pinheiro, H.A.; Totola, M.R.; Loureiro, M.E. Photochemical responses and oxidative stress in two clones of Coffea canephora under water deficit conditions. *Environ. Exp. Bot.* **2002**, *47*, 239–247. [CrossRef]
32. Liu, F.; Jensen, C.R.; Shahanzari, A.; Andersen, M.N.; Jacobsen, S.E. ABA regulated stomatal control and photosynthetic water use efficiency of potato (*Solanum tuberosum* L.) during progressive soil drying. *Plant Sci.* **2005**, *168*, 831–836. [CrossRef]
33. Goodarzian-Ghahfarokhi, M.; Mansouri-Far, C.; Saeidi, M.; Abdoli, M. Different physiological and biochemical responses in maize hybrids subjected to drought stress at vegetative and reproductive stages. *Acta Biol. Szeged.* **2016**, *60*, 27–37.
34. Yadav, S.; Sharm, K.D. Molecular and Morphophysiological Analysis of Drought Stress in Plants. In *Plant Growth*; Rigobelo, E.C., Ed.; IntechOpen: London, UK, 2016; pp. 149–173. [CrossRef]
35. Acevedo, E.; Hsiao, T.C.; Henderson, D.W. Immediate and subsequent growth responses of maize leaves to changes in water status. *Plant Physiol.* **1971**, *48*, 631–636. [CrossRef] [PubMed]
36. Chen, D.; Wang, S.; Cao, B.; Cao, D.; Leng, G.; Li, H.; Yin, L.; Shan, L.; Deng, X. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Front. Plant Sci.* **2016**, *6*, 1–15. [CrossRef] [PubMed]
37. Farre, I.; Oijen, M. van, Leffelaar, P.A.; Faci, J.M. Analysis of maize growth for different irrigation strategies in northeastern Spain. *Eur. J. Agron.* **2000**, *12*, 225–238. [CrossRef]
38. Gaffney, J.; Schussler, J.; Löffler, C.; Cai, W.; Paszkiewicz, S.; Messina, C.; Groeteke, J.; Keaschall, J.; Cooper, M. Industry-Scale Evaluation of Maize Hybrids Selected for Increased Yield in Drought-Stress Conditions of the US Corn Belt. *Crop Sci.* **2015**, *55*, 1608–1618. [CrossRef]
39. Catsky, J. Determination of water deficit in disks cut out from leaf blades. *Biol. Plantarum.* **1960**, *2*, 76–78. [CrossRef]
40. Strasser, B.J.; Strasser, R.J. Measuring Fast Fluorescence Transients to Address Environmental Questions: The JIP-test. In *Photosynthesis: From Light to Biosphere*; Mathis, P., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995; pp. 977–980.
41. Strasser, R.J.; Srivastava, A.; Tsimilli-Michael, M. The Fluorescence Transient as a Tool to Characterize and Screen Photosynthetic Samples. In *Probing Photosynthesis: Mechanism, Regulation and Adaptation*, 1st ed.; Yunus, M., Pathre, U., Mohanty, P., Eds.; CRC Press: Boca Raton, FL, USA, 2000; pp. 445–483.
42. Silvestre, S.; Araújo, S.; Vaz Patto, M.C.; Marques da Silva, J. Performance index: An expeditious tool to screen for improved drought resistance in the *Lathyrus* genus. *J. Integr. Plant Biol.* **2014**, *56*, 610–621. [CrossRef]
43. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Method. Enzymol.* **1987**, *148*, 350–382. [CrossRef]
44. Feijão, E.; Gameiro, C.; Franzitta, M.; Duarte, B.; Caçador, I.; Cabrita, M.T.; Matos, A.R. Heat wave impacts on the model diatom Phaeodactylum tricornutum: Searching for photochemical and fatty acid biomarkers of thermal stress. *Ecol. Indic.* **2017**, *95*, 1026–1037. [CrossRef]
45. Gameiro, C.; Utkin, A.B.; Cartaxana, P.; Marques da Silva, J.; Matos, A.R. The use of laser induced chlorophyll fluorescence (LIF) as a fast and non-destructive method to investigate water deficit in Arabidopsis. *Agri. Water Manag.* **2016**, *164*, 127–136. [CrossRef]
46. Thirunavukkarasu, N.; Hossain, F.; Arora, K.; Sharma, R.; Shiriga, K.; Mittal, S.; Mohan, S.; Namratha, P.M.; Dogga, S.; Rani, T.S.; et al. Functional mechanisms of drought tolerance in subtropical maize (Zea mays L.) identified using genome-wide association mapping. *BMC Genom.* **2014**, *15*, 1182. [CrossRef] [PubMed]
47. Nascimento, R.; Maia, M.; Ferreira, A.E.; Silva, A.B.; Freire, A.P.; Cordeiro, C.; Silva, M.S.; Figueiredo, A. Early stage metabolic events associated with the establishment of *Vitis vinifera*—Plasmopara viticola compatible interaction. *Plant Physiol. Biochem.* **2019**, *137*, 1–13. [CrossRef]
48. Hellemans, J.; Mortier, G.; De Paepe, A.; Speleman, F.; Vandesompele, J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol.* **2007**, *8*, R19. [CrossRef] [PubMed]
49. Chiango, H.; Figueiredo, A.; Sousa, L.; Sinclair, T.; Marques da Silva, J. Assessing drought tolerance of traditional maize genotypes of Mozambique using chlorophyll fluorescence parameters. *S. Afr. J. Bot.* **2021**, *138*, 311–317. [CrossRef]
50. Cruz de Carvalho, R.; Cunha, A.; Marques da Silva, J. Photosynthesis by six Portuguese maize cultivars during drought stress and recovery. *Acta Physiol. Plant.* **2011**, *33*, 359–374. [CrossRef]

51. Min, H.; Chen, C.; Wei, S.; Shang, X.; Sun, M.; Xia, R.; Liu, X.; Hao, D.; Chen, H.; Xie, Q. Identification of drought tolerant mechanisms in maize seedlings based on transcriptome analysis of recombination inbred lines. *Front. Plant Sci.* **2016**, *7*, 1–19. [CrossRef]
52. Franks, P.J.; Farquhar, G.D. The effect of exogenous abscisic accid on stomatal development, tomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. *Plant Physiol.* **2001**, *125*, 935–942. [CrossRef] [PubMed]
53. Harb, A.; Krishnan, A.; Ambavaram, M.M.R.; Pereira, A. Molecular and physiological analysis of drought stress in arabidopsis reveals early responses leading to acclimation in plant growth. *Plant Physiol.* **2010**, *154*, 1254–1271. [CrossRef] [PubMed]
54. Marques da Silva, J.; Arrabaça, M.C. Photosynthesis in the water stressed C4 grass Setaria sphacelata is mainly limited by stomata with both rapidly and slowly imposed water deficits. *Physiol. Plant* **2004**, *121*, 409–420. [CrossRef]
55. Bjorkman, O.; Demmig, B. Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* **1987**, *170*, 489–504. [CrossRef] [PubMed]
56. Epron, D.; Godard, D.; Cornic, G.; Genty, B. Limitation of net CO2 assimilation Rate by internal resistances to CO2 Transfer in the leaves of 2 tree species (*Fagus sylvatica* L and *Castanea sativa* Mill). *Plant Cell Environ.* **1995**, *18*, 43–51. [CrossRef]
57. Kalaji, H.M.; Govindjee; Bosa, K.; Kościelniak, J.; Zuk-Gołaszewska, K. Effects of salt stress on photosystem II efficiency and CO2 assimilation of two Syrian barley landraces. *Environ. Exp. Bot.* **2011**, *73*, 64–72. [CrossRef]
58. Correia, P.M.; da Silva, A.B.; Roitsch, T.; Carmo-Silva, E.; Marques da Silva, J. Photoprotection and optimization of sucrose usage contribute to faster recovery of photosynthesis after water deficit at high temperatures in wheat. *Physiol. Plant.* **2020**, 1–14. [CrossRef]
59. Chandrasekar, V.; Sairam, R.K.; Srivastava, G.C. Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. *J. Agron. Crop. Sci.* **2000**, *185*, 219–227. [CrossRef]
60. Aranjuelo, I.; Molero, G.; Erice, G.; Avice, J.C.; Nogués, S. Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.). *J. Exp. Bot.* **2011**, *62*, 111–123. [CrossRef]
61. Pintó-Marijuan, M.; Munné-Bosch, S. Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: Advantages and limitations. *J. Exp. Bot.* **2014**, *65*, 3845–3857. [CrossRef] [PubMed]
62. Kitajima, K.; Hogan, K.P. Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant Cell Environ.* **2003**, *26*, 857–865. [CrossRef] [PubMed]
63. Chaves, M.M.; Maroco, J.P.; Pereira, J.S. Understanding plant responses to drought—From genes to the whole plant. *Funct. Plant Biol.* **2003**, *30*, 239–264. [CrossRef] [PubMed]
64. Upchurch, R.G. Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnol. Lett.* **2008**, *30*, 967–977. [CrossRef] [PubMed]
65. Gigon, A.; Matos, A.R.; Laffray, D.; Zuily-Fodil, Y.; Pham-Thi, A.T. Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (Ecotype Columbia). *Ann. Bot-London* **2004**, *94*, 345–351. [CrossRef] [PubMed]
66. Matos, A.R.; Gigon, A.; Laffray, D.; Pêtres, S.; Zuily-Fodil, Y.; Pham-Thi, A.T. Effects of progressive drought stress on the expression of patatin-like lipid acyl hydrolase genes in Arabidopsis leaves. *Physiol. Plant.* **2008**, *134*, 110–120. [CrossRef]
67. Sebastiana, M.; da Silva, A.B.; Matos, A.R.; Alcântara, A.; Silvestre, S.; Malhó, R. Ectomycorrhizal inoculation with *Pisolithus tinctorius* reduces stress induced by drought in cork oak. *Mycorrhiza* **2018**, *28*, 247–258. [CrossRef]
68. Dakhma, W.S.; Zarrouk, M.; Cherif, A. Effects of drought-stress on lipids in rape leaves. *Phytochemistry* **1995**, *40*, 1383–1386. [CrossRef]
69. Seiwert, D.; Witt, H.; Ritz, S.; Janshoff, A.; Paulsen, H. The Nonbilayer Lipid MGDG and the Major Light-Harvesting Complex (LHCII) Promote Membrane Stacking in Supported Lipid Bilayers. *Biochemistry* **2018**, *57*, 2278–2288. [CrossRef]
70. Los, D.A.; Murata, N. Membrane fluidity and its roles in the perception of environmental signals. *Biochim. Biophys. Acta Biomembr.* **2004**, *1666*, 142–157. [CrossRef] [PubMed]
71. Duarte, B.; Matos, A.R.; Caçador, I. Photobiological and lipidic responses reveal the drought tolerance of *Aster tripolium* cultivated under severe and moderate drought: Perspectives for arid agriculture in the mediterranean. *Plant. Physiol. Biochem.* **2020**, *154*, 304–315. [CrossRef] [PubMed]
72. Zheng, G.; Tian, B.; Zhang, F.; Tao, F.; Li, W. Plant adaptation to frequent alterations between high and low temperatures: Remodeling of membrane lipids and maintenance of unsaturation levels. *Plant Cell Enviro.* **2011**, *34*, 1431–1442. [CrossRef] [PubMed]
73. Zhong, D.; Du, H.; Wang, Z.; Huang, B. Genotypic variation in fatty acid composition and unsaturation levels in bermudagrass associated with leaf dehydration tolerance. *J. Am. Soc. Hortic. Sci.* **2011**, *136*, 35–40. [CrossRef]
74. Loll, B.; Kern, J.; Saenger, W.; Zouni, A.; Biesiadka, J. Towards complete cofactor arrangement in the 3.0 A resolution structure of photosystem II. *Nature* **2005**, *438*, 1040–1044. [CrossRef] [PubMed]
75. Camoni, L.; Di Lucente, C.; Pallucca, R.; Visconti, S.; Aducci, P. Binding of phosphatidic acid to 14-3-3 proteins hampers their ability to activate the plant plasma membrane H+-ATPase. *IUBMB Life* **2012**, *64*, 710–716. [CrossRef] [PubMed]
76. Chen, L.; Cao, B.; Han, N.; Tao, Y.; Zhou, S.F.; Li, W.C.; Fu, F.L. Phospholipase D family and its expression in response to abiotic stress in maize. *Plant. Growth. Regul.* **2017**, *81*, 197–207. [CrossRef]
77. Zhao, J. Phospholipase D and phosphatidic acid in plant defence response: From protein-protein and lipid-protein interactions to hormone signalling. *J. Exp. Bot.* **2015**, *66*, 1721–1736. [CrossRef]

78. An, Z.F.; Li, C.Y.; Zhang, L.X.; Alva, A.K. Role of polyamines and phospholipase D in maize (Zea mays L.) response to drought stress. *S. Afr. J. Bot.* **2012**, *83*, 145–150. [CrossRef]
79. Hayano-Kanashiro, C.; Calderón-Vásquez, C.; Ibarra-Laclette, E.; Herrera-Estrella, L.; Simpson, J. Analysis of gene expression and physiological responses in three Mexican maize landraces under drought stress and recovery irrigation. *PLoS ONE* **2009**, *4*, e7531. [CrossRef]
80. Bellon, M.R.; van Etten, J. Climate Change and On-farm Conservation of Crop Landraces in Centres of Diversity. In *Plant Genetic Resources and Climate Change*; Jackson, M., Ford-Lloyd, B., Parry, M., Eds.; CAB International: Wallingford, UK, 2014; pp. 137–150.
81. Ficiciyan, A.; Loos, J.; Sievers-Glotzbach, S.; Tscharntke, T. More than Yield: Ecosystem Services of Traditional versus Modern Crop Varieties Revisited. *Sustainability* **2018**, *10*, 2834. [CrossRef]