Maize Male Reproductive Organs Affected by Different Timings of Water Deficit

Yebei Li
China Agricultural University
Chunjiang Wu
China Agricultural University
Bingchao Zhang
China Agricultural University
Ping Zhang
China Agricultural University
Pu Wang
China Agricultural University
Shoubing Huang (huangshoubing@cau.edu.cn)
China Agricultural University

Research Article

Keywords: pollen vitality, ultra-structure, maize, water deficit

Posted Date: August 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-775794/v1

Abstract

Compared with female reproductive organs, the development of male reproductive organs was got less attention in maize because of its oversupply in amount even under water deficit. Thus, a rainout shelter experiment was designed to explore the effect of different timings of water deficit on pollen vitality and exterior and interior ultra-structure of pollen grains, starch particles in pollen grains, anther fresh weight, and vascular bundle number and its organizational structure in tassel pedicel. There were five water treatments included in this study, *viz.* well water treatment (CK), water deficit during 6- to 8- leaf stage (V_{6-8}), 9- to 12- leaf stage (V_{9-12}), 13-leaf stage to tasseling (V_{13-T}), and silking to blister (R_{1-2}), respectively. Results showed that the percentage of pollen grains with strong vitality decreased remarkably by 27.3–45.9% under water deficits, while that of pollen grains with weak vitality increased by 27.2–34.7%. The percentage of pollen grains with no vitality was significantly increased only when water deficit occurred around silking, which was up to 8.6% for V_{13-T} and 19.7% for R_{1-2} compared with 1.0% for that of CK. Both shrunken pollen apertures (including annulus and operculum) and less starch particles might partially explain the weakened pollen vitality for water deficits before tasseling. Furthermore, the assimilation flux to male reproductive organs might be restricted by the influenced vascular bundle system under water deficits before tasseling, with manifestation showing in anther fresh weight and starch particle status in pollen grains. Specifically, V_{9-12} and V_{13-T} water deficits delayed differentiation of vascular bundle but had no influence on vascular bundle number, which might be one reason for their decreased anther fresh weight and less starch particles in pollen grains. Conversely, V_{6-8} water deficit significantly decreased vascular bundle number but had no significant influence on anther fresh weight and starch particles in pollen grains. R_{1-2} water deficit almost had no influences on above indicators except for pollen vitality. Overall, this research highlight that male reproductive organs could be influenced by water deficits in maize, which deserves more attention in further breeding especially under the background of high-quality requirement for pollen vitality of the maize hybrids that have a small tassel size.

Introduction

In maize, female reproductive organs were more prone to get fatal threat under water stress, while male reproductive organs were usually neglected mainly because of its oversupply also less influence by water deficits[1-4]. A large tassel with more branches could cause redundant assimilation consumption and resource competition with female reproductive organs, even though it is benefit to the successful fertilization[5,6]. In order to reduce unnecessary resource competition, maize varieties with a small tassel had been regarded as one important breeding direction in the previous breeding programs[7-9]. In this way, a higher requirement for pollen vitality is essential since male reproductive organs were more vulnerable under unfavorable environment. Water deficit as one of the most threat abiotic stresses for crop production, it tends to be more uncertain for its occurring timing and more serious for its severity with climate change[10]. Thus, it is of great importance to explore the response of male reproductive organs to

water deficit in maize, which could guide farmers and agronomist to better avoid the influence on final grain yield.

Water deficit at around flowering could cause a great yield reduction in maize[11-14]. This decreased grain yield had be demonstrated to be mainly resulted from decreased kernel number, which was associated with the decreased amount and vitality of reproductive organs[1,2,15-17], asynchronous flowering time[18-20], and zygotic abortion[4,21-23]. Among these, male organs were seldomly regarded as the limitation for grain yield reduction in maize[15,24,25]. However, male organs played a crucial role for fertilization in cleistogamy crops, like rice (*Oryza sativa*) and wheat (*Triticum aestivum* L.)[26-31]. In these crops, male failure under abiotic stress might result from failed male gametophyte[32], invalid anther dehiscence[33], and weakened pollen vitality[34], etc. In maize, previous researches showed water deficit around flowering had limited effects on pollen vitality[2,15,24], which might be due to (a) water deficit timing that was not overlapped with pollen sensitive period (during meiosis and mitosis) and (b) oversupply of pollen grains that might compensate for poor pollen vitality. However, there were still some documents proposed that water deficit could have direct effects on male organ growth, like, delaying male development[12], decreasing pollen fertility[35] and pollen available amount[25,36]. Moreover, a small tassel is more favorable when breeding drought-tolerant maize hybrids that are likely more sensitive to adverse environment[9,37]. In this way, it will propose a high requirement for pollen vitality in the context of increasingly severe water deficit.

The decreased pollen vitality was related to its changed structure[38,39], inner contents[40,41], and endogenous phytohormones[42,43]. Specifically, pollen abortion could be triggered by abnormal degradation of tapetum[41,44], disturbed mitochondrial metabolism[45,46], and abnormal dissolution of callose walls[47]. Meanwhile, the disturbed sugar metabolism in male reproductive organ was regarded as one reason for pollen sterile in several crops under water deficit[34,44,48]. Anther development and pollen maturing dependent on the photoassimilate from the source tissues. Leaf productivity could be reduced by water deficit because of stoma closure[49] and the weakened photosynthetic system[50,51]. The vascular bundle system that connects source and sink is essential for assimilate transportation and allocation, which was decreased by water deficit in both number and size[52]. The strength of sink could also have a feedback to assimilation production of source tissue[53]. Thus, the balance of “source-flow-sink” system is of great importance for male reproductive development[54,55].

Compared to cleistogamous crops, there were few researches focusing on male reproductive organ development in maize even under water deficits. In this study, we focused on male reproductive organ development under different timings of water deficit, and explored the effect of water deficits on pollen vitality, and the possible underlying accessories. Exterior and interior ultra-structure of pollen grains, anther fresh weight, vascular bundle number and its organizational structure in tassel pedicel were observed around tasseling to explain their relationship with pollen vitality under water deficit.

Materials And Methods

Plant growing and water regimes

Hybrid maize Zhengdan 958 was used as material in this study. Plants were growing in cement sealed ponds under rainout shelter during 2014-2016 at Shangzhuang Experimental Station, China Agricultural University (Beijing, China), as shown in Li *et al*.[52] . Plants were thinned at the 3-leaf stage and then plant density was established with 0.6 m row spacing and 0.2 m plant spacing. In order to meet the nutrient requirement for plant growth, N (60 kg ha^{-1}), P (90 kg P_2O_5 ha^{-1}), K (150 kg K_2O ha^{-1}) were evenly scattered on the soil surface, and then plowed into soil by shovels before planting. Top-dressing was proceeded at the 12-leaf stage through furrowing application with 120 kg ha^{-1} N. During maize growth season, cutworm (*Agrotis segetum*) and corn borers (*Ostrinia nubilalis*) were controlled by applying phoxim at the 3-leaf stage and carbofuran at the 10-leaf stage, respectively. Weeds were removed artificially when it is necessary. The plant material employed in the present is in compliance with relevant institutional, national, and international guidelines and legislation.

Five irrigation regimes were arranged with a completely randomized design, including four water deficit treatments and one well-irrigated treatment. Five-times irrigation processed during maize growth season were at 6-leaf stage (V_6, the 1st irrigation), 9-leaf stage (V_9, the 2nd irrigation), 13-leaf stage (V_{13}, the 3rd irrigation), tasseling (R_1, the 4th irrigation) and blister (R_2, the 5th irrigation), respectively, for well-irrigated treatment. Water deficits were achieved by omitting irrigation once at 6-leaf stage for $V_{6\text{-}8}$, at 9-leaf stage for $V_{9\text{-}12}$, at 13-leaf stage for $V_{13\text{-}T}$, and at tasseling for $R_{1\text{-}2}$, respectively. Irrigation was applied manually by hose. The irrigation amount was calculated separately for every 20 cm soil layer (0-20 cm, 20-40 cm, and 40-60 cm) and then summed up, as shown in Li *et al*.[52]. The equation for irrigation amount (I) of each layer was I = 1000 BD·D·A (80% FD−SWC), where BD means soil bulk density, D means soil layer depth, A means plot area, FD means field capacity, and SWC means soil water content. The soil water content increased up to 80% of field capacity after irrigation, while it dropped down to approx. 50% of field capacity during water deficit period[52]. Shelter was covered whenever it rained before the end of all water deficit treatments (R2), and then it kept opening afterward. The meteorological data were collected from China Meteorological Administration (http://data.cma.cn/site/index.html), showing averaged temperature and sunshine hours during water deficit periods (Supplementary Table 1).

Sampling for pollen grains and TTC staining for pollen vitality

Fresh pollen grains were collected from each treatment whenever tassel is blooming in 2016. Target tassels were shaken gently to remove old pollen grains before sampling. And then fresh pollen grains were collected by gently taping tassels with a container covered by a fine sieve screen below. These sampled pollen grains were put into plastic bags with exhausted air, and then transferred to the lab immediately with an iced container.

Pollen vitality was detected by TTC (2,3,5-triphenyltetrazolium chloride) staining with 0.5% (w/v) TTC solution dissolved by 0.1 M PH=7.0 sodium phosphate buffer ($NaHPO_4$-NaH_2PO_4). Fresh pollen grains

were dropped on a slide with a small spoon and mixed with one drop of TTC solution by a pipette. Specimens were covered by the coverslips and put in the dark over 15 minutes, and then observed and took microphotos under stereoscope (Olympus SZ60, Olympus Imaging China Co., Ltd., Beijing, China) immediately. At least 5 fields of view were selected for statistics and computing percentage of each type of pollen grains. Pollen grains were classified into 3 types according to its displayed color after TTC staining. Specifically, pollen grains in dark red were regarded with strong vitality, in light red with weak vitality, and in ivory with no vitality, respectively. Percentage of different types of pollen grains was calculated through the number of each type of pollen grains divided by the number of all observed pollen grains.

Anther fresh weight measurement and organizational structure observation of vascular bundles in tassel pedicel

Five tassels from each treatment were collected at around tasseling (55 days after planting) in 2016, and then stored at -80℃ freezer before measurement. All three strong anthers were extracted by dissecting needle from fifteen stalked florets located at middle tassel inflorescence, followed by fresh weight measurement immediately (Fig. 3).

Three tassels collected at 3 days after the 3rd irrigation (49 days after planting) in 2016 were used for organizational structure observation. The observed segments were about 1 cm-long sections cut from the basal tassel pedicel through single blades. Subsequently, samples were put into formaldehyde acetic acid solution (FAA) composed of 10% formasldehyde, 50% ethanol, and 5% acetic acid immediately and stored at 4℃ before paraffin section proceeding. After a series of dehydrated and infiltrated, tassel pedicel segments were finally embedded in paraffin wax as showed in Li *et al.*[52]. Slices with thickness of 4 μm were acquired through Leica RM 2016 microtome (Leica Shanghai Instrument Co., Ltd. Shanghai, China). Safranin-fast green (0.5%, w/v) staining were used for slices coloring. Finally, micrographs were taken by Nikon Elipse Ci (Nikon Instruments Inc., Shanghai, China), and analyzed by Case Viewer (3DHISTECH Ltd., Utah, Hungary).

Ultra-structure observation of exterior and interior pollen grains

Part of the above collected fresh pollen grains in each treatment were saved in two 2 ml centrifuge tubes filled with 2.5% glutaraldehyde dissolved by PH=7.4 0.1M PBS (sodium phosphate buffer, $NaHPO_4$-NaH_2PO_4). Pollen grains from one centrifuge tube was used for exterior ultra-structure observation through scanning electron microscope (Hitachi S-3400 N, Toyko, Japan) after pretreatment and coated with gold palladium, as mentioned in Wang *et al.*[56]. Pollen grains for interior ultra-structure observation was through transmission electron microscope (JEM-1230, Tokyo, Japan), which also referred to the method mentioned in Wang *et al.*[56].

Pollen area was the average areas of 25-30 pollen grains in 2015 and 27-54 pollen grains in 2016, which was measured with Image Pro Plus 6.0. The annulus and operculum area of pollen apertures were the

mean value of 3-4 pollen grains in 2015 and 4-8 pollen grains in 2016 were recorded.

Data analysis

Significant analysis was progressed using SAS 9.0 (SAS Institute 2004). Analysis of variance was determined using the least significant differences at a probability level of 0.05. Figures were drawn with SigmaPlot 12.5 (Systat Software Inc. 2013).

Results

Pollen vitality under water deficit

As shown in Fig. 1, results displayed that well-irrigated treatment (CK) had the highest percentage of pollen grains with high vitality (61.5%), while the percentages of pollen grains with weak (37.5%) and no (1.0%) vitality were the lowest, compared to water deficit treatments (Fig. 1). V_{6-8} and V_{9-12} treatments hold similar percentages in all three types of pollen grains (Fig. 1A-B, C-D). In detail, the percentages of pollen grains with a high vitality decreased down to 34.2% for V_{6-8} and 31.8 % for V_{9-12}, while the percentages of pollen grains with weak vitality increased up to 64.9% for V_{6-8} and 65.3% for V_{9-12}, as compared to that of CK. There was no significant difference observed for the percentages of pollen grains with no vitality among V_{6-8}, V_{9-12} and CK. For V_{13-T} and R_{1-2} treatments, the percentages of pollen grains with a high vitality decreased significantly down to 19.2% for V_{13-T} and 15.6% for R_{1-2}, while the percentages of pollen grains with both weak and no vitality increased significantly (Fig. 1A-B, E-F). Specifically, the percentages of pollen grains with a weak vitality were 72.2% for V_{13-T} and 64.7% for R_{1-2}, and percentages of pollen grains with no vitality was 8.6% for V_{13-T} and 19.6% for R_{1-2}.

Size and ultra-structure of pollen grains under water deficit

The size of pollen grains had been influenced by water deficits especially during V_9-R_1 (Fig. 2 A_1-D_1, F_1 and F_2). Significant decrease in pollen area was shown for V_{9-12} and V_{13-T} in 2015 and for V_{9-12} during 2016, but increased for V_{6-8} in 2016, compared to CK treatment. There was no significant difference among CK, V_{6-8}, and R_{1-2} in 2015, and among CK, V_{13-T}, and R_{1-2} in 2016 (Fig. 2 A_1-E_1, F_1 and F_2).

Although there were no consistent results between two experimental years, the annulus area of pollen aperture were prone to be shrinking after water deficits (Fig. 2A_2-E_2, G_1 and G_2). Specifically, the annulus area of pollen aperture had a trend to be smaller with the delay of pre-flowering water deficits, with a remarkable decrease in V_{6-8}, V_{9-12}, and V_{13-T} compared to CK during 2015 (Fig. 2 A_2-D_2, G_1 and G_2). The annulus area of pollen aperture were not significantly different between CK and R_{1-2} in both 2015 and 2016 (Fig. 2 A_2, E_2, G_1 and G_2).

Regarding to the operculum area of pollen aperture, significant decrease in V_{13-T} in 2015 and V_{9-12} in 2016 was observed, compared to CK (Fig. 2 H_1 and H_2).

Anther fresh weight and starch particles in pollen grains under water deficit

As shown in Fig. 3, the longer anthers from 15 florets were pooled together for anther fresh weight measurement. Results showed that anther fresh weight significantly decreased in $V_{9\text{-}12}$ and $V_{13\text{-}T}$ treatment but was not affected in $V_{6\text{-}8}$ and $R_{1\text{-}2}$ treatment, compared to CK (Fig. 3 A). In addition, anther fresh weight decreased greater for later water deficits prior to flowering, with the most decrease in $V_{13\text{-}T}$.

The effect of water deficit on starch particles in pollen grains were observed through transmission electron microscope (TEM), as shown in Fig. 4. With the same magnification, starch particles were more and bigger in pollen grains from well-irrigated treatment than that from water deficit treatments. Starch particles in pollen grains from $V_{9\text{-}12}$ and $V_{13\text{-}T}$ had obviously less number than other treatments. Furthermore, starch particles seemed to concentrate along one side of pollen grain wall for CK and $V_{9\text{-}12}$ but scattered more evenly in pollen grains from $V_{6\text{-}8}$, $V_{13\text{-}T}$, and $R_{1\text{-}2}$ treatments, which was because of uncontrolled crossing position when slicing.

Cross section and the vascular bundle number in tassel pedicel under water deficit

Vascular bundle number in tassel pedicel and its organizational structure were showed in Fig. 5. Water deficits significantly decreased the number of vascular bundles only for $V_{6\text{-}8}$ water deficit compared to well-watered treatment (Fig. 5 A-C). The size of vascular bundle showed smaller in water deficit treatments than that of CK treatment, even though there was similar number of vascular bundles among $V_{9\text{-}12}$, $V_{13\text{-}T}$ and CK (Fig. 5 A-E).

Discussion

In this study, maize pollen grains with weak vitality increased due to water deficits, while that with no vitality increased the most in $R_{1\text{-}2}$ water deficit, followed by $V_{13\text{-}T}$, and with no difference between $V_{9\text{-}12}$ and $V_{6\text{-}8}$ water deficit and CK. As a consequence, water deficit imposed around flowering hold the lowest amount of pollen grains that with high vitality (Fig. 1). Pollen grains and anthers were in developmental progression before tasseling, both of which were sensitive to unfavorable environments and were more easily influenced[35,57,58]. Water deficit had a greater influence on the size of pollen grains, grain aperture and anther weight nearly prior to tasseling (Fig. 2, 3). During the period from V_{13} to tasseling, male reproductive organ develops, including pollen grain size, pollen aperture and, and starch condition, a sensitive growth period to water deficit[35,59,60], which can explain the smallest anther fresh weight in $V_{13\text{-}T}$ water deficit (Fig. 3). The response of vascular bundle system could be one reason for the reduced anther fresh weight in pre-tasseling water deficit (Fig. 3, 5). Moreover, the reduced starch particles in pollen grains might also contribute to the low fresh weight of anthers (Fig. 3, 4). Differently, $R_{1\text{-}2}$ water deficit had no influence on pollen characteristics, since pollen grains and anthers almost matured at around tasseling (Fig. 1-4). The difference in pollen vitality between $R_{1\text{-}2}$ water deficit and CK might be related to the plant water status that was caused by the irrigation regime in this study. Soil moisture of $R_{1\text{-}2}$ kept

dropping since irrigation at 13-leaf stage (V13) till the next water supply at around 10 days after silking. However, there were documents indicating that the water potential of reproductive organs was prone to be sustained or less influenced despite an appreciable decrease in leaf water potential[2,35]. Thus, the indirect consequence of discrepancy in water status of plants might contribute to the different performance in pollen vitality between these two treatments such as ABA that can be regarded as a transportable sporicidal signal[35,56].

The development of male reproductive organs was tightly associated with the assimilate flux through vascular bundle system that connecting productive and sink tissues[61]. Vascular bundle in tassel pedicel was as the terminal of assimilation flux that supporting male inflorescence, which was of great essential for the assimilation amount. In this study, there were differences in the amount and status of vascular bundles in tassel pedicel among treatments during processing, even they were observed far away from its final matured status as mentioned above (Fig. 5). Thus, the differentiation of vascular bundle largely depended on the growth stage of the whole plants. Pollen vitality as the final manifestation of male organ development was influenced by a series of developmental processes. Pollen vitality was closely associated with pollen structure per se. Mature pollen grains with a strong vitality were usually fully hydrated also with a shape of inflated prolate spheroid, while pollen grains were prone to be shrank after losing vitality[62]. In our study, there was no obvious surficial structure damage by all water deficits, but shrinking size in both pollen grains itself and pollen apertures existed especially for $V_{9\text{-}12}$ and $V_{13\text{-}T}$ water deficits (Fig. 2). The percentage of pollen grains with no vitality was less affected by these two water deficits, but the percentage of pollen grains with weak vitality had been significantly increased (Fig. 1). Thus, we deduced that this shrinking pollen grains and pollen apertures by these water deficits might not have a fatal damage for pollen grains but it might associate with the weak vitality of pollen grains. Then, the development of anthers-the carrier of pollen grains is also of great essential for pollen maturation. In our study, water deficits at $V_{9\text{-}12}$ and $V_{13\text{-}T}$ had an irreversible effect on the fresh weight of anthers that carried with smaller pollen grains (Fig. 2 and 3). In rice, the abnormal programmed cell death happened for anther walls after suffering water deficit, which had a detrimental effect on pollen vitality[32]. Additionally, starch content and its related sugar metabolism inside pollen grains also influenced pollen vitality[28,34,63]. In this study, pollen grains from $V_{9\text{-}12}$ and $V_{13\text{-}T}$ water deficits had less starch particles, which could explain the increased percentage of pollen grains with weak and no vitality (Fig 1 and 4). Even though water deficit could decrease leaf photosynthesis ability during and after water stress, the non-reducing sugar in pollen still increased[52,64]. Moreover, the starch in pollen grains decreased, which suggested that not carbon starvation but the process from substrate to starch was obstructed in pollen grains[28,65]. Also, Sheoran and Saini[28] showed that the invertase and starch synthase in anthers played an important role in restraining the utilization of assimilation and starch synthesis. Furthermore, the assimilation flow might be restrained by delayed development of vascular bundle in tassel pedicle in this study, as showed in Fig. 5. There was also report indicating that decreased starch in sterile pollen was accompanied with poorly developed vascular bundle system[61]. Additionally, weak pollen viability was also related to the lower moisture content because of the increased vapor pressure deficit (VPD) between

air and pollen itself after exposure in air for several hours[62,66]. Overall, influenced pollen vitality was a comprehensive results of a series factors after maize suffering water deficits.

Pollen vitality was influenced by water deficits, in which pollen grains with high vitality decreased the most under water deficits around flowering in this study (Fig. 1). This result seemed to be inconsistent with previous results[15,24,67]. The inconsistency could be explained by the different approaches used for pollen vitality testing. The reciprocal cross pollination approach that was used in previous studies could make the weak pollen vitality obscured due to the oversupply of pollen grains, while TTC staining could directly detect the vitality of each individual pollen grains[68]. The influenced pollen vitality had no fatal effect on kernel setting under water deficits, even with 14.0-19.1% kernel number losing observed for water deficits around flowering[52]. One hand, the poor quality of the pollen grains could be compensated by the great amount of pollen grains. There are over millions of pollen grains produced by each tassel, while there are maximum approx. 1000 silks for individual ear need to be fertilized[36]. The other hand, documents had clarified other more important factors associated with this kernel number reduction under water deficits in maize, like, asynchronous flowering, silk arrest, and kernel abortion[4,16,17,22].

As mentioned above, the detecting method for pollen vitality will affect the judgement for its effect on kernel setting. Several methods including direct and indirect approaches were usually used in one experiment to improve measurement precision[69-71]. Merits and demerits usually coexisted in each approach. In-vitro media culture has been regarded as a more precise and reliable approach, in which pollen vitality is determined through calculating pollen germination percentage, but a high possibility of pollen bursting was the drawback of this approach[72,73]. Thus, it is urgent and necessary to develop a more efficient and burst-avoided cultivation medium. Also, pollen germination could also be observed in vitro through fluorescence after aniline blue staining[29,74]. Dye staining reaction as another direct testing approach is faster and easier to operate but sometimes prone to overestimate pollen vitality[75]. The available dyes for detection include 2,3,5-triphenyltetrazolium chloride (TTC) for redox reaction, iodine/potassium iodide for starch staining (I_2-KI), and isatin for proline staining[76,77]. Pollen vitality could also be determined through seed set counting after pollination with the tested pollen grains[77], which is a relatively accurate approach and widely used by agronomist. But the equivalent amount of pollen grains is difficult to be determined in this approach.

Conclusion

This study clarified that the development of male reproductive could be affected by water deficits in maize, which is worthy of more attention in further studies. Specifically, weakened pollen vitality had been observed under water deficits especially around flowering. There was also no fatal damage for pollen grains, but the shrunken pollen aperture and pollen grains itself might be associated with its weakened pollen vitality for water deficits imposed before tasseling. Less starch particles displayed in pollen grains from V_{9-12} and V_{13-T} water deficits, which could partly explain the decreased anther fresh weight also the weakened pollen vitality. Furthermore, the decreased amount of vascular bundle in tassel pedicel

under water deficits may potentially limit the flux of assimilation to pollen grains. Additionally, R_{1-2} water deficit had no influence on above mentioned pollen characteristics except for pollen vitality indicating there are other reasons for the decreased pollen vitality in water deficit near flowering.

Declarations

Author Contributions

Y.L. and P.W. designed this experiment, Y.L., C.W. and B.Z. conducted the field and lab experiments, Y.L. analyzed the data, Y.L. and S.H. wrote the manuscript, all authors reviewed the manuscript.

Acknowledgments

We deeply appreciate the financial supporting from National Science Foundation of China (grant No. 31571592), and China Scholarship Council (grant No. 201606350211).

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Figures

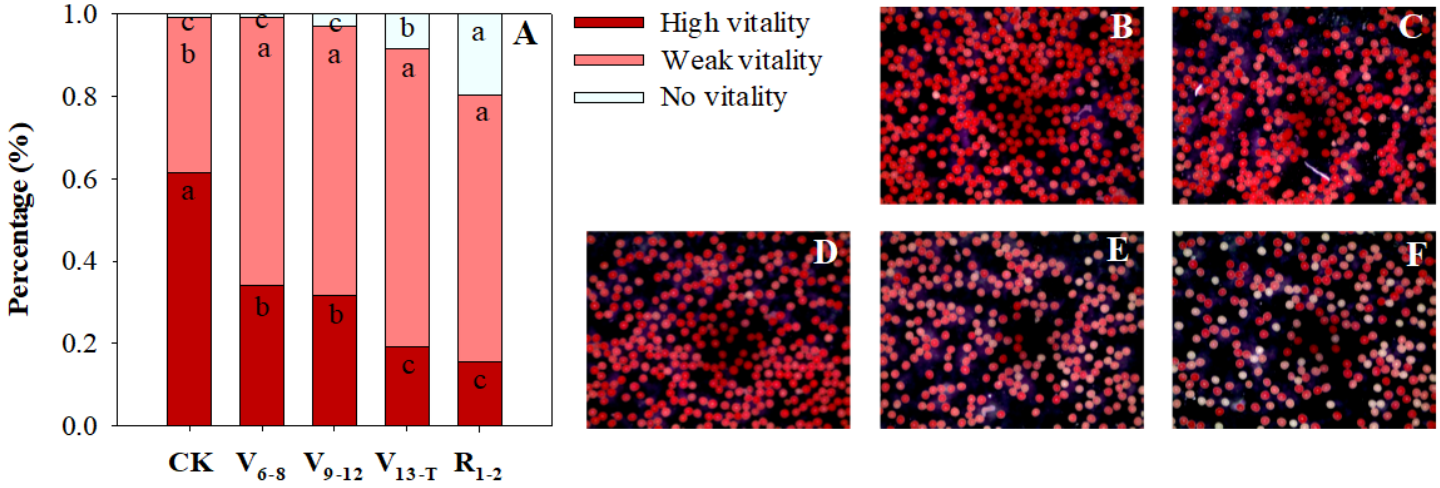

Figure 1

The percentage of different types of pollen grains (A) and TTC staining slices of pollen grains (B-F) under well-irrigated and water deficit treatments during 2016. CK, well-irrigated treatment, V6-8, water deficit during 6- to 8-leaf stage, V9-12, water deficit during 9- to 12-leaf stage, V13-T, water deficit during 13-leaf stage to tasseling, R1-2, water deficit during silking to blister. Pollen grains in dark red means strong vitality, in light red means weak vitality, in ivory means no vitality. Different letters among treatments within same type of pollen grains represent significant difference at $p<0.05$ in the histogram.

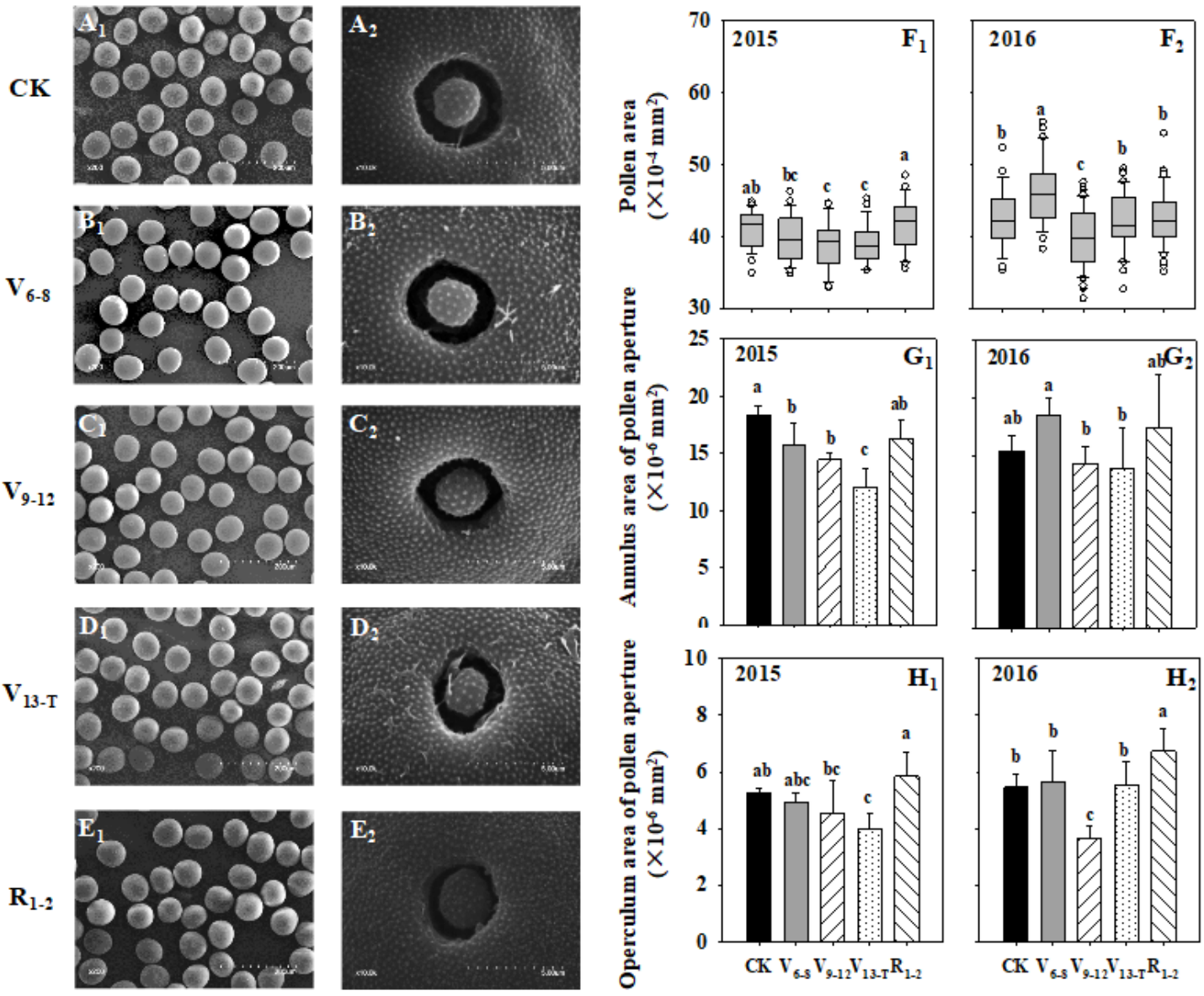

Figure 2

Scanning electron micrographs of pollen grains (A1-E1) and pollen aperatures (A2-E2) during 2015, and the area of pollen grains (F1-F2), annulus (G1-G2) and operculum (H1-H2) of pollen aperture under well-irrigated and water deficit treatments during 2015-2016. CK, well-irrigated treatment, V6-8, water deficit during 6- to 8-leaf stage, V9-12, water deficit during 9- to 12-leaf stage, V13-T, water deficit during 13-leaf stage to tasseling, R1-2, water deficit during silking to blister. Different letters among treatments represent significant difference at $p<0.05$ in the histogram.

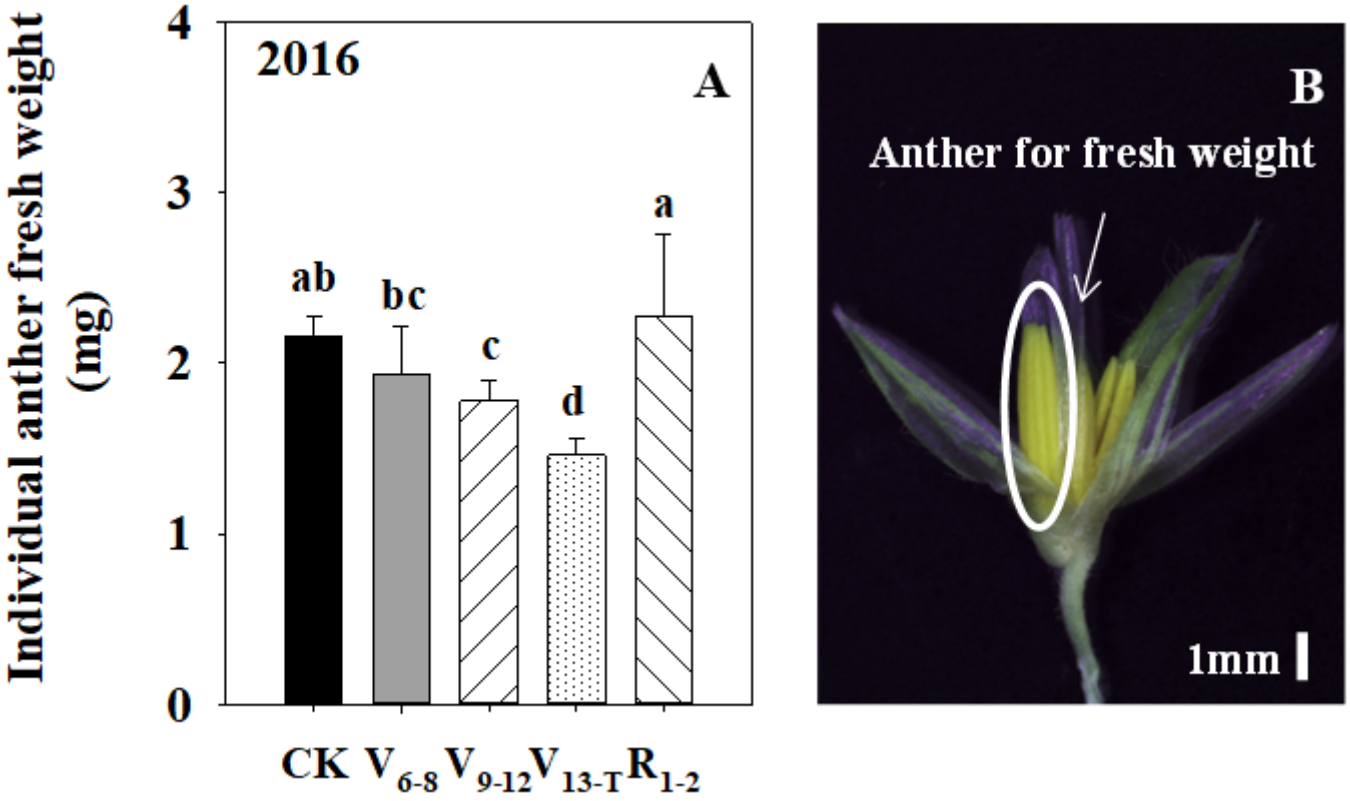

Figure 3

Individual anther fresh weight under well-irrigated and water deficit treatments during 2016 (A) and the selected anthers for fresh weight showed in B. CK, well-irrigated treatment, V6-8, water deficit during 6- to 8-leaf stage, V9-12, water deficit during 9- to 12-leaf stage, V13-T, water deficit during 13-leaf stage to tasseling, R1-2, water deficit during silking to blister. Different letters among treatments represent significant difference at $p<0.05$ in the histogram.

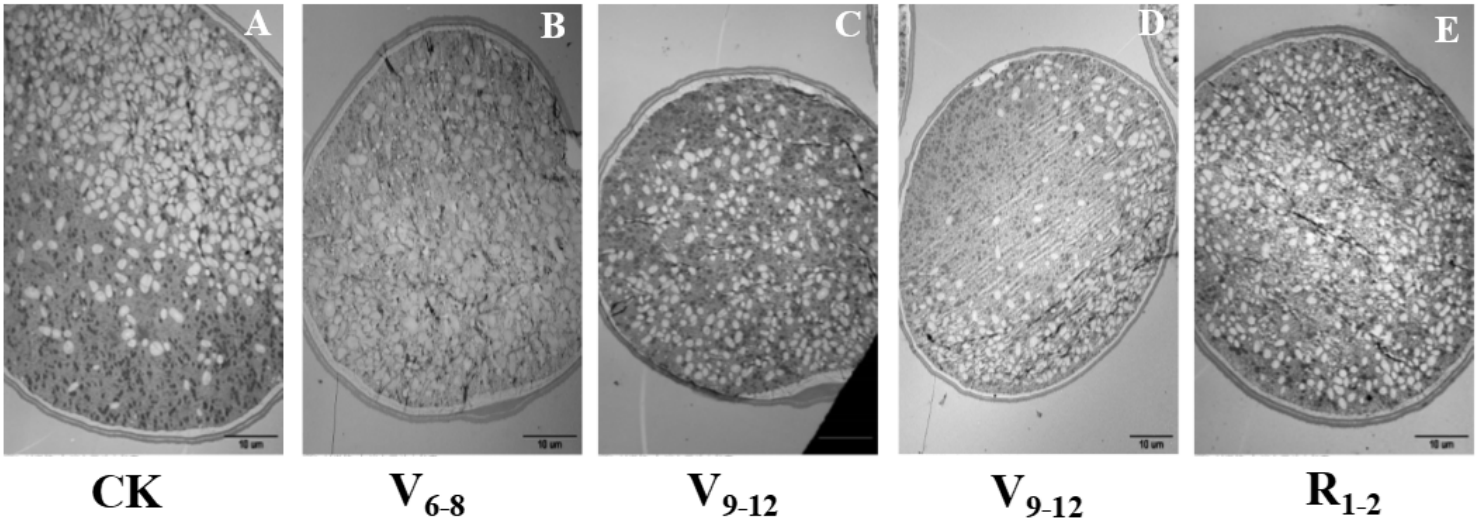

Figure 4

Interior ultra-structure of pollen grains under well-irrigated and water deficit treatments collected at around tasseling in 2015. CK, well-irrigated treatment, V6-8, water deficit during 6- to 8-leaf stage, V9-12, water deficit during 9- to 12-leaf stage, V13-T, water deficit during 13-leaf stage to tasseling, R1-2, water deficit during silking to blister.

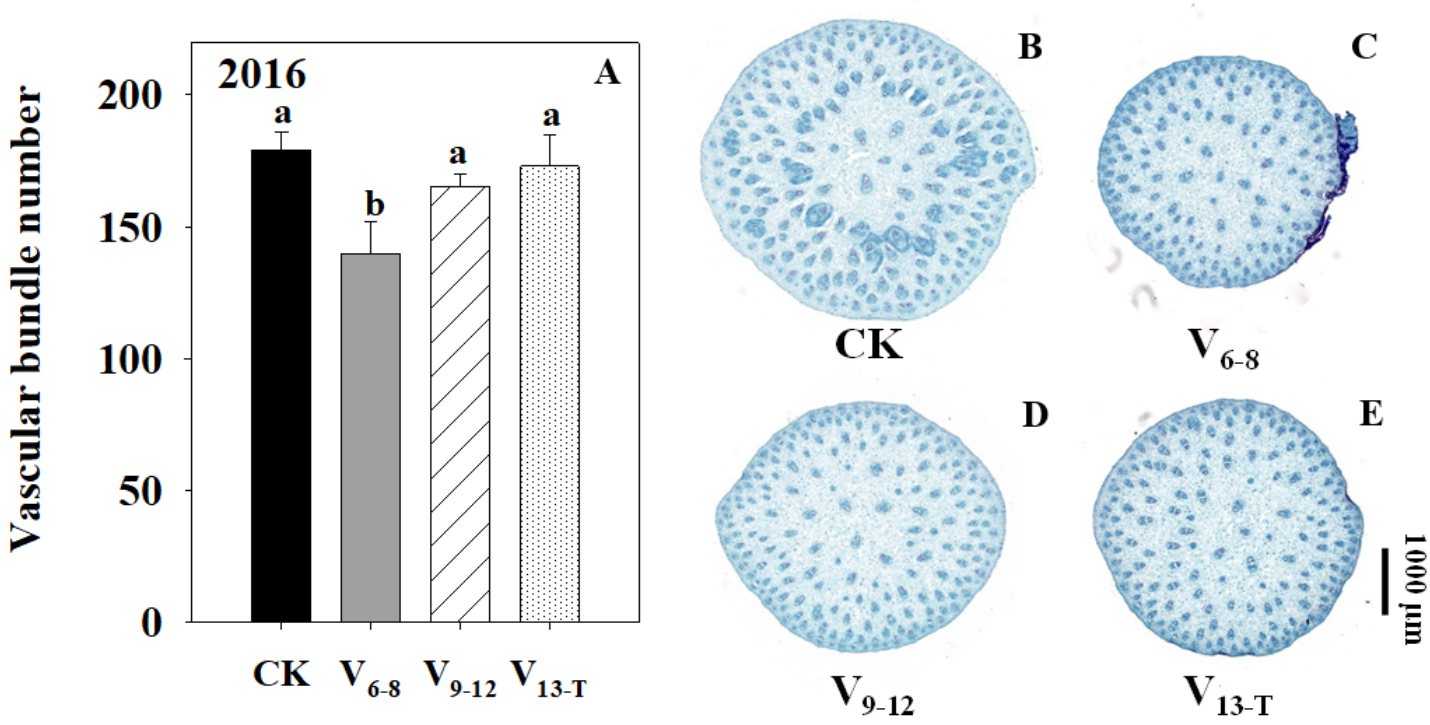

Figure 5

Vascular bundle number (A) and its micrographs (B-E) at 49 days after planting (DAP) under well-irrigated and water deficit treatments during 2016. CK, well-irrigated treatment, V6-8, water deficit during 6- to 8-leaf stage, V9-12, water deficit during 9- to 12-leaf stage, V13-T, water deficit during 13-leaf stage to tasseling, R1-2, water deficit during silking to blister. Different letters among treatments represent significant difference at $p<0.05$ in the histogram.

Supplementary Files

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