Original Research Article

Correlation studies on pollen attributes and grain yield under drought stress in a wild multiparental population of rice (*Oryza sativa* L.)

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Abstract

The drought stress at the reproductive stage is causing a drastic reduction in rice yield. This is mainly due to the failure of male gametogenesis or dysfunction of male gametophyte. The wild *Oryza* species found to have more alleles contributing to drought tolerance and can be integrated to cultivated species. So, in this investigation, the change in pollen parameters with response to drought in the reproductive stage was studied in a wild introgressed multiparental population. The genotypes like WRM 27-48-4, WRM 74-9-4 and WRM 107-21-2 were identified from the population with minimum yield loss also had less reduction in the pollen parameters.The mean reduction in the yield of these genotypes was less than the cultivated Asian parent, which is drought susceptible. The correlation studies revealed that the pollen parameters are significantly and directly contributing to the seed yield under drought conditions. This suggests that the selection based on these characters will help to identify the genotypes which can reduce the yield loss due to reproductive stress.

Key words: Rice, Drought, Pollen fertility, Pollen length, Pollen surface area, wild *Oryza*

Introduction

Rice in one of the important staple food crops and the major energy source of the fifty per cent of the global population(Tagliapietra, Soares, and Clerici 2024). The demand of rice is increasing with the rapidly growing global population. Attaining higher productivity in rice to meet the global demand in this era of drastic climate change is a challenging task for the crop scientists.The temperature extremes, the erratic precipitation and other climatic irregularities will highly affect the crop production directly and indirectly. The projected climate change studies predict the decrease in the rice yield by 2.11 per cent by 2040 in the global level despite of its increasing demand(Li et al., 2024).Rice crop found to have mixed effects as responses to climate change.So to overcome the situation and to sustain the rice production, intensive crop improvement programs should be adopted.

The rice crop has already reached its plateau of improvement due to its narrow genetic base. So, to broaden the genetic base and to increase the variability of rice, its wild genetic diversity can be explored. The wild *Oryza* species is having several favorable alleles for increasing the productivity as well as to fight against the biotic and abiotic stresses(Bhatia et al. 2017). All the A genome species in the primary gene pool areinter-crossable and can be directly used for crop improvement programs(Sanchez, Wing, and Brar 2013).To increase the maximum variability and crossing overs, multiparental populations are preferred over biparental crosses(Cavanagh et al. 2008).

Rice, as a semi aquatic crop, needs more water for its growth. The high yielding cultivars of present day works well in the high water input systems and not adapted to the rainfed systems. The high yielding varieties with more water use efficiency and can tolerate the water stress is essential for the sustainability in rice production. The varying climate is highly imposing several stresses to the crop growth. Drought is one of such important stress which is estimated to be affecting more than 50 percentage of the rice producing areas and limiting the rice production adversely(Kumar et al., 2023; Li & Xu, 2007). The unavailability of drought tolerant high yielding varieties worsens the situation(Kamarudin et al. 2018).

The drought stress can affect all the critical growth stages of rice and is more severe at the stages of germination and reproduction. Reproductive stage of rice is very sensitive to the external stimuli and any stress in this growth phase will cause a heavy yield loss (Salgotra and Chauhan 2023). The water scarcity will cause detrimental effects on anther dehiscence and pollen fertility (Rang et al. 2011). The water scarcity in the male gametogenesis stage will results in viability loss in pollen mainly because of the inability of mitochondria to meet the energy demands of the functional pollen development. The stress causes the rise in ROS and decrease in antioxidant enzymes which will cause programmed cell death leading to fertilization failure and eventually the yield loss (Nguyen et al. 2009).

The resilience in the anther dehiscence and the maintenance of pollen size during water stress are two major traits that makes a rice variety drought tolerant(Liu et al. 2006). Several metabolic changes during drought affect the pollen development and viability during drought.

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The drought alters several signalingpathways (like auxin, ABA, ROS, jasmonic acid etc.), which affect the metabolic processes during pollen development. It detrimentally affects the cell division, carbohydrate availability, expression of sugar transporter genes etc. which will lead to drought injuries (Huang et al. 2024). The different studies on the effect of drought stress on reproductive phase and pollen development of rice is summarized in Table 1.

Considering this background, the experiment was focused on finding the correlation of the pollen characters with grain yield in drought stress and control conditions in wild multiparental advance generation intercross (MAGIC) population of rice.

Materials and methods

The introgressed lines from $F_{4:5}$generation of wild rice multiparental advance generation intercross (MAGIC) constituted the materials for study. The MAGIC population is developed from an eight parental intercross involving two cultivated and six wild species belonging to the A genome diploid species viz., *O. sativa* (NSICRc 222), *O. glaberrima* (CG14), *O. rufipogon* (Acc. 106286), *O. barthii* (Acc. 100936), *O. nivara* (Acc. 101508) *O.glumaepatula* (Acc. 105692), *O.longistaminata* (Acc. 110404) and *O. meridonalis* (Acc. 105290).

The cultivated parents (*O. sativa* (NSICRc 222), *O. glaberrima* (CG14)) along with 100 introgression lines were raised in rain-out shelterof Department of Rice in Tamil Nadu Agricultural University, Coimbatore (11° 00’ N latitude, 77° 00’ E longitude) to screen for drought in reproductive stage. Each entry was sown directly in the wet soil of the rain-out shelter. Two rows of each entry were sown with a spacing of 20 cm between the rows and between the entries. Irrigation was given along with the cultural practices and plant protection measures till 67th day from the date of sowing. Then the irrigation was withheld for the rest of the growth period except the life irrigation. The pollen sterility and the physiological parameters were recorded during the stress period.The control plot was maintained for comparison in which the recommended package of practices for cultivation and irrigation schedule was maintained.The following pollen parameters were observed

Pollen fertility

The 10 florets from the primary tiller were taken and pollen sterility was studied at the heading stage. The pollen grains were collected in the glass slides from the florets by squeezing

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2-3 anthers by using a needle. Staining was done with 1 per cent Iodine-Potassium iodide solution. The count of stained (fertile) and unstained (sterile) pollen grains was taken for 3 microscopic fields. And pollen fertility was calculated using the formula:

$$Pollen\ sterility = \frac{Number\ of\ fertile\ pollen\ grains}{Total\ number\ of\ pollen\ grains} \times 100$$

Pollen length (μm)

A randomly selected 5 pollen grains in each field were used for measurement of pollen length. The pollen length was measured under a binocular microscope (Software: ScopeImage 9.0.exe).

Pollen radius (μm)

A randomly selected 5 pollen grains in each field were used for measurement of pollen radius (μm). The above parameters were measured under a binocular microscope (Software: ScopeImage 9.0.exe).

Pollen surface area (μm^2)

A randomly selected 5 pollen grains in each field were used for measurement of pollen surface area (μm^2). The above parameters were measured under a binocular microscope (Software: ScopeImage 9.0.exe).

Results and discussion

The experiment revealed that the pollen parameters are highly affected by the drought stress. The change in pollen parameters and the single plant yield is demonstrated in Table 2. The genotypes like WRM 27-48-4, WRM 74-9-4 and WRM 107-21-2 which had least reduction in yield also had less reduction in the pollen parameters. Several studies suggestthat there is adverse effect of drought and temperature stress that will directly affects the crop yield (Fahad et al. 2018; Feng et al. 2018; Salgotra and Chauhan 2023).

The genotypes those found to have reduction in pollen parameters will eventually affect the crop yields. Water availability in the reproductive stage affects the overall development of the pollen(Rao et al. 2018). The genotypes with minimum reduction in the pollen parameters during

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water stress could be resistant to reproductive drought stress.The resilience in the anther dehiscence and the maintenance of pollen size during water stress are two major traits that determine drought tolerance in the reproductive stage(Liu et al. 2006).The drought stress will induce several irreversible effects in the male reproductive parts which interrupt several proteins for anther and pollen development. These effects were found to be irreversible and more severe in susceptible genotypes. The redox imbalance caused by the drought stress severely affect the pollen parameters leading to spikelet sterility(Kumar et al. 2023; Liu and Bennett 2011).

The mean reduction of yield of the introgressed lines (5.64) was less than that of the cultivated Asian parent (28.94). The pollen parameters were also showing the same trend proving their superiority to withstand the reproductive stress. The parental species of wild rice MAGIC population like *O. nivara, O. rufipogon and O. glumaepatula* are the proven doors for drought tolerance which are having several signaling pathways which imparts tolerance to water stress (Kondamudi *et al.*, 2016). Some high protein genes in *O.nivara* introgression lines found to impart better drought tolerance by reducing the pollen sterility due to drought(Puteh et al. 2013; Saragih et al. 2013).

The correlation analysis (Table 3 and Table 3) revealed that, even though there was no much effect of pollen characters on seed yield and seed set percentage in non-stress condition, in drought stress, they are highly influencing the seed yield. Pollen length is found to have highest significant correlation with the single plant yield. All the pollen parameters are having direct positive correlation with the pollen fertility. The earlier studies suggest that the pollen development is a determinant factor for the pollen sterility. The drought alters several signalingpathways (like auxin, ABA, ROS, jasmonic acid etc.), which affect the metabolic processes during pollen development. It detrimentally affects the cell division, carbohydrate availability, expression of sugar transporter genes etc. which will lead to drought injuries(Yu, Jiang, and Guo 2019). The excessive accumulation of ROS during stress cause tapetal specific programmed cell death during pollen development which also contribute to pollen sterility(Zhao et al. 2023)

The pollen length, radius and surface area are significantly contributing to seed yield per plant. These characters are the indicators of pollen hydration which directly affect the reproductive success during drought(Covey et al. 2010). These indicate that the resistant cultivars have the

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capacity to maintain pollen hydration even in water stress condition.The pollen hydration and dehydration are controlled by several transcription factors which directly affects the pollen germination and the double fertilization process(Sze et al., 2024; Zhao et al., 2023).The pollen radius and pollen surface area affect the sporopollenin deposition and also the fertilization (AbdElgawad et al. 2015).

The pollen fertility is not found to have a correlation with percent seed set and yield which are contrary to studies ofSharma et al., 2018and Rao et al. (2018). So, from this, it is found that there are some factors other than pollen fertility in this introgression lines which affect the fertilization success other than pollen viability which can be explored further.

Conclusion

The variation of the pollen parameters, seed set and the seed yield in drought stress vary according to genotypes. The wild introgressions increased the tolerance to drought stress by influencing the pollen parameters. These pollen parameters like pollen length, radius and surface area have positive correlation with the seed set and the seed yield under drought stress. So improvement in these characters can be utilized for the development of drought tolerant genotypes. And the superior introgression lines form this population can be utilized as donors for such characters which can overcome reproductive stage drought.

Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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Table 1. Effect of drought stress on reproductive stage and pollen development in rice

Stage affected	Effect	Reference
Reproductive stage	Yield loss	Salgotra and Chauhan (2023)
Reproductive stage	Redox imbalance Spikelet sterility	Kumar et al. (2023)
Reproductive stage	Ill effects on cell division and metabolic pathways	Yu et al. (2019)
Reproductive stage	Effects on Pollen hydration and dehydration, double fertilization	Sze et al., (2024); Zhao et al. (2023)
Anther dehiscence	Pollen sterility	Rang et al. (2011)
Male gametogenesis	Pollen sterility Yield loss	Nguyen et al., 2009
Anther dehiscence & Pollen development	Decrease in pollen size Pollen sterility	Liu et al. (2006)
Pollen development	Oxidative stress and change in metabolic pathways	Huang et al. (2024)
Pollen development	Pollen architecture	Rao et al. (2018)

Table 2. Differences in pollen parameters, percent seed set and the yield per plant due to drought stress

Sr. No.	Genotypes	PFDD	PLDD	PRDD	PSADD	PSSD	YLDD
1.	WRM 2-12-5	3.53	0.05	0.45	25.36	0.55	6.89
2.	WRM 3-35-5	55.39	0.78	0.99	37.15	5.28	7.58
3.	WRM 5-26-3	2.21	1.17	0.05	0.1	6.89	4.39
4.	WRM 6-7-1	76.57	3.57	1.78	74.14	0.75	5.65
5.	WRM 6-21-4	11.83	0.86	0.4	18.01	1.95	6.01
6.	WRM 17-9-2	12.94	0.9	0.58	28.49	0.86	3.37
7.	WRM 17-46-2	1.13	0.1	0.2	0.55	0.33	4.02
8.	WRM 21-16-4	4.71	0.81	0.83	41.19	6.64	10.13
9.	WRM 21-24-1	43.07	2.38	0.58	22.07	4.72	2.93
10.	WRM 23-25-5	3.25	0.01	0.35	19.81	1.14	2.69
11.	WRM 23-47-3	1.92	0.19	0.18	0.85	0.39	2.58
12.	WRM 24-35-2	4.47	0.1	0.04	0.72	6.83	13.85
13.	WRM 24-48-2	13.13	1.63	0.76	26.13	1.38	2.41
14.	WRM 25-17-2	21.62	1.14	0.26	11.15	18.12	4.06
15.	WRM 26-2-3	1.18	0.95	1.61	71.59	7.05	1.16
16.	WRM 27-24-1	8.5	3.29	1.26	34.58	2.22	6.12
17.	WRM 27-48-4	1.43	0.09	0.1	0.12	0.94	1
18.	WRM 29-33-3	2.71	0.78	0.18	6.27	6.24	2.39

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Sr. No.	Genotypes	PFDD	PLDD	PRDD	PSADD	PSSD	YLDD
19.	WRM 29-39-2	9.1	1.9	0.55	22.48	3.68	5.28
20.	WRM 30-6-2	5.34	0.83	1.04	45.79	0.90	8.09
21.	WRM 30-12-2	0.91	0.3	0.02	2.82	1.89	2.06
22.	WRM 31-20-4	1.41	2.35	0.37	19.35	0.64	12.5
23.	WRM 31-21-5	3.91	1.1	0.44	9.02	0.27	2.58
24.	WRM 32-34-4	3.97	0.27	0.23	9.54	6.54	1.2
25.	WRM 32-40-3	0.89	0.24	0.04	1.32	0.97	2
26.	WRM 35-7-1	1.29	0.47	0.09	0.44	6.47	2.17
27.	WRM 35-12-1	0.88	0.25	0.21	8.16	2.00	2.15
28.	WRM 36-5-4	3.51	0.18	0.29	7.68	0.10	1.45
29.	WRM 36-21-2	9.01	0.53	0.14	8.72	0.11	3
30.	WRM 37-1-4	2.67	0.14	0.05	0.27	1.38	1.4
31.	WRM 38-1-3	0.74	0.03	0.03	0.28	2.29	14.71
32.	WRM 38-34-5	6.65	0.6	0.25	1.17	0.59	8.38
33.	WRM 40-15-2	9.92	0.06	0.58	4.09	15.06	1.91
34.	WRM 41-14-5	2.96	0.1	0.21	3.86	0.49	2.68
35.	WRM 41-20-2	2.49	0.05	0.18	9.15	0.03	6.95
36.	WRM 42-15-2	73.24	3.61	1.23	40.17	0.66	5.94
37.	WRM 42-71-1	16.27	0.92	0.63	25.64	5.55	3.53
38.	WRM 44-40-1	1.65	0.38	0.25	16.11	14.19	2.14
39.	WRM 44-48-5	2.92	0.96	0.01	7.37	6.69	3.3
40.	WRM 50-27-5	23.86	0.57	0.32	8.91	1.03	1.5
41.	WRM 50-45-2	3.4	1.35	0.2	9.62	11.58	2.33
42.	WRM 52-9-5	1.62	0.38	0.14	0.83	11.16	39.58
43.	WRM 52-22-5	0.93	0.8	0.08	18.85	1.08	4.9
44.	WRM 54-30-1	2.08	0.51	0.15	13.01	0.57	3.26
45.	WRM 54-40-3	5.39	1.7	0.19	6.45	0.77	5.85
46.	WRM 55-22-5	3.52	1	0.64	28.48	0.67	4.29
47.	WRM 58-4-1	12.45	0.74	0.43	10.34	2.33	16.04
48.	WRM 58-17-4	0.6	0.97	0.15	6.78	1.01	25.92
49.	WRM 59-7-3	2.06	0.09	0.24	7.6	11.32	1.95
50.	WRM 61-1-5	8.7	0.93	0.32	8.23	4.75	3.98
51.	WRM 61-16-5	1.29	1.6	0.58	25.19	0.95	1.38
52.	WRM 70-18-3	3.34	1.15	0.19	9.97	0.91	5.34
53.	WRM 70-22-2	6.63	1.05	0.08	4.22	3.88	2.07
54.	WRM 72-4-4	1.68	0.65	0.03	0.52	5.46	4.06
55.	WRM 72-15-1	1.13	0.68	0.15	4.29	7.23	4.92
56.	WRM 73-6-1	3.63	0.01	0.12	3.32	2.44	2.84
57.	WRM 73-38-4	4.63	0.17	0.09	1.39	9.90	5.9

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Sr. No.	Genotypes	PFDD	PLDD	PRDD	PSADD	PSSD	YLDD
58.	WRM 74-9-4	3.05	0.23	0.15	7.81	96.54	0.84
59.	WRM 74-14-1	3.31	0.67	2.81	0.87	0.84	2.75
60.	WRM 76-13-5	1.16	0.22	0.16	2.5	3.36	1.24
61.	WRM 76-48-5	5.65	0.41	0.22	3.91	1.30	15.49
62.	WRM 78-2-3	0.67	0.44	0.19	5.47	1.86	8.38
63.	WRM 78-19-1	0.48	0.09	0.19	0.1	0.30	2.47
64.	WRM 80-23-1	1.62	0.19	0.5	8.25	6.32	2.87
65.	WRM 80-24-2	0.8	0.38	0.01	2.61	3.50	1.14
66.	WRM 88-38-3	2.86	0.56	0.13	6.45	0.92	2.72
67.	WRM 88-40-1	0.71	0.51	0.15	2.37	1.85	5.97
68.	WRM 89-34-4	29.98	0.98	0.71	20.97	0.31	1.02
69.	WRM 89-44-2	4.7	0.51	0.1	5.09	4.92	4.7
70.	WRM 92-1-2	1.89	0.8	0.33	6.69	5.09	2.39
71.	WRM 92-4-1	44.11	1.14	0.68	10.76	2.71	1.46
72.	WRM 93-20-3	2.63	0.51	0.07	2.12	4.05	12.31
73.	WRM 93-39-5	9.47	0.42	0.27	2.82	0.43	2.38
74.	WRM 95-4-2	11.62	1.59	0.33	7.19	7.74	6.13
75.	WRM 95-25-5	7.84	1.49	0.29	8.8	5.00	4.48
76.	WRM 97-13-5	4.77	0.49	0.39	13.47	1.02	4.14
77.	WRM 99-16-3	1.3	0.46	0.08	2.43	13.98	1.73
78.	WRM 99-41-1	4.27	0.29	0.31	7.84	7.72	40.78
79.	WRM 101-14-4	13.61	0.31	0.18	3.49	14.60	6.02
80.	WRM 103-7-1	1.15	0.1	0.2	1.2	0.95	7.98
81.	WRM 105-14-1	2.47	0.23	0.48	7.86	20.73	3
82.	WRM 105-17-4	22.63	1.04	0.42	19.62	21.00	2.82
83.	WRM 106-7-5	3.11	0.36	0.08	3.76	9.91	2.01
84.	WRM 106-8-5	39.53	0.56	0.03	6.97	0.91	2.04
85.	WRM 107-8-1	3.83	0.2	0.35	19.35	4.46	5.51
86.	WRM 107-21-2	1.28	0.25	0.14	0.83	1.00	0.47
87.	WRM 108-9-2	3.6	0.11	0.17	4.23	8.25	28.87
88.	WRM 109-27-5	2	0.94	0.32	18.01	9.92	4.21
89.	WRM 110-1-2	4.14	0.45	0.05	0.59	4.03	6.1
90.	WRM 110-14-3	1.57	0.01	0.25	1.93	1.91	1.83
91.	WRM 112-7-2	1.86	0.07	0.23	0.2	7.09	1.94
92.	WRM 112-13-2	4.47	1.07	0.39	15.57	15.98	1.39
93.	WRM 114-3-1	2.04	0.3	0.25	1.87	2.52	2.95
94.	WRM 116-5-4	2.48	0.01	0.25	2.41	2.90	3.66
95.	WRM 116-37-5	1.63	0.3	0.13	9.13	1.04	2.32
96.	WRM 117-14-4	4.52	0.01	0.06	2.4	6.70	3.86

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Sr. No.	Genotypes	PFDD	PLDD	PRDD	PSADD	PSSD	YLDD
97.	WRM 117-15-1	1.03	0	0.17	4.51	8.59	2.7
98.	WRM 119-7-5	9.53	0.24	0.42	3.09	1.10	2.85
99.	WRM 120-1-5	6.95	1.13	0.46	0.49	3.91	15.92
100.	WRM 124-25-1	25.25	0.44	0.3	1.6	4.35	13.48
101.	*O. sativa* var. NSICRc222	15.94	1.64	1.15	20.06	1.02	28.94
102.	*O. glaberrima* var.CG14	1.03	0.21	0.34	2.05	1.92	1.99
	Mean	**8.17**	**0.68**	**0.34**	**10.87**	**5.14**	**5.64**

PFDD-Difference in Pollen fertility(%), PLDD-Difference in pollen length(μm), PRDD-Difference in pollen radius(μm) PSADD-Difference in pollen surface area(μm^2), PSSD-Difference in per cent seed set(%), YLDD-Difference in yield per plant(g)

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Table 3. Correlation between the pollen characters and seed yield under non stress condition

	PFC	PLC	PRC	PSAC	PSSC	YLDC
PFC	1	-.297**	-0.187	-.224*	.462**	-0.015
PLC		1	.862**	.865**	-0.167	0.131
PRC			1	.971**	-0.087	0.093
PSAC				1	-0.092	0.106
PSSC					1	0.118
YLDC						1

PFC-Pollen fertility (Control), PLC-Pollen length (Control), PRC-Pollen radius (Control), PSAC-Pollen surface area (Control), PSSC-Percent seed set (Control), YLDC-Single plant yield (Control).

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

Table 4. Correlation between the pollen characters and seed yield under drought stress condition

	PFD	PLD	PRD	PSAD	PSSD	YLDD
PFD	1	0.231*	0.239*	0.241*	0.03	0.115
PLD		1	0.797**	0.850**	0.085	0.319**
PRD			1	0.862**	0.065	0.220*
PSAD				1	0.108	0.250*
PSSD					1	0.199*
YLDD						1

PFC-Pollen fertility (Drought), PLC-Pollen length (Drought), PRC-Pollen radius (Drought), PSAC-Pollen surface area (Drought), PSSC-Percent seed set (Drought), YLDC-Single plant yield (Drought)

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

Figures

Figure 1. Variation of pollen fertility of $F_{4:5}$ wild rice MAGIC progenies under drought stress

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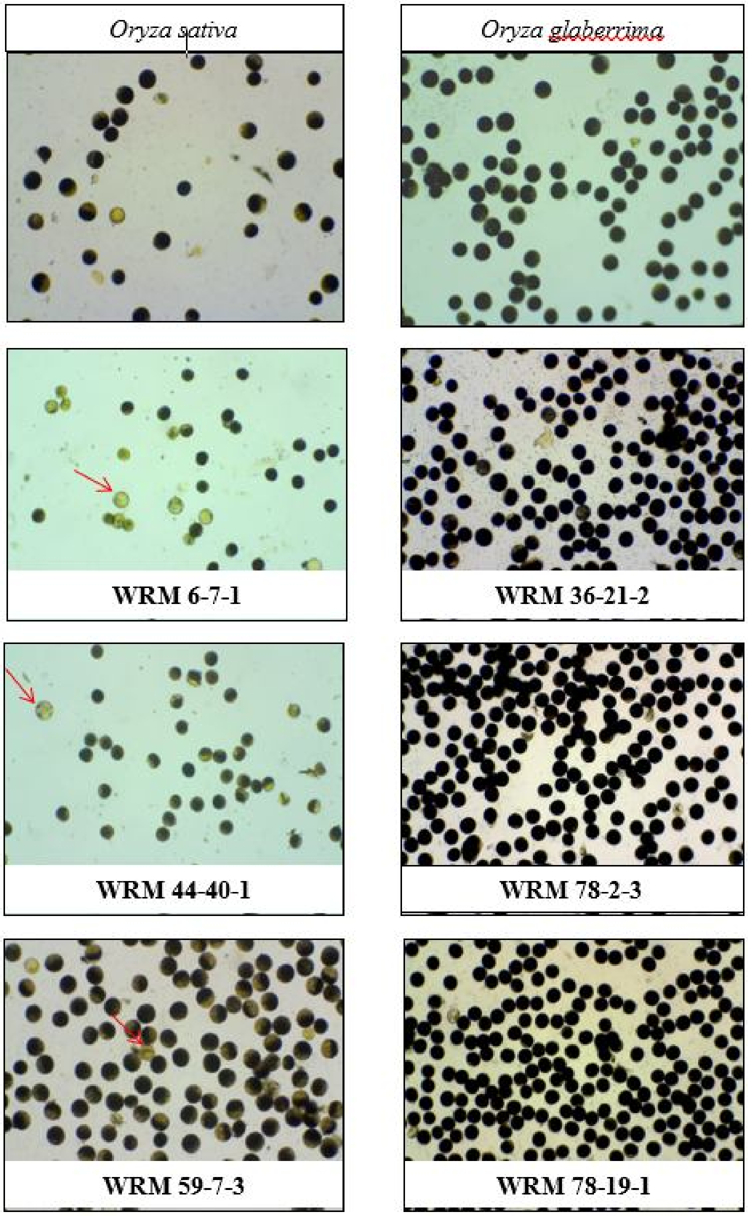

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Figure 2. Variation in pollen parameters of $F_{4:5}$ wild rice MAGIC progenies under drought stress