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STUDIES ON SOME FACTORS AFFECTING GRAIN SET PERCENTAGE IN CONTROLLED CROSSES OF RICE

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ABSTRACT

Grains of the old conservative rice cv. Agamy (Sabini) were sown in greenhouse during the two successive summer seasons 1999 and 2000. The aim of this study was to find out the factors that may affect success of controlled crosses in rice cv. Agamy (Sabini) The factors affecting grain set percentages under study were pollen age, stigma receptivity and time (hour) of pollination. Results indicated that pollens over two hours old resulted in a low grain set percentage and pollen viability was greatly affected by the time elapsed since its excursion from the anthers. Pollen viability was greatly decreased as the pollen age increased. Six and twelve hour's pollen ages showed a considerable reduction in grain set percentages.

Stigmas of 1 or 2 days old resulted in the highest grain set percentage. Stigmas of age over 5 days showed a comparable low grain set percentages. Stigma receptivity was greatly affected by its age (the time elapsed since its emergence from the florets) and the proper age for stigmas to receive the pollen was one to two days.

The period ranging between early morning 6 A.M. to mid morning 9 A.M. showed the high mean percent of grain set. Pollination hour after 10 A.M. greatly resulted in a decrease in mean percent of grain set. This reduction reached its maximum at noon hour or later on.

INTRODUCTION

In Egypt the old traditional conservative rice cultivar Agamy (Sbaini) possesses many desirable agronomic characters such as early maturity, salt tolerance and cooked fluffy but it lacks most favorable yield characters. Practically in breeding programs this variety almost fails in hybridization due to low grain set.

Factors controlling hybrid grain yield in rice and many other crops are numerous. Temperature and relative humidity at booting affected glume opening and pollen viability (Wang *et al*., 1988 and 1993), Sensitivity to water deficiency was also reported (Ekanayake *et al*., 1990), Salinity at either panicle initiation or the booting stage reduced pollen viability (Khatun and Flowers 1995). An association was noted between the anther and pollen grain traits on the one hand and components of grain yield on the other (Chowdhry *et al.,* 1994). Variety used as a parent affects the percent of grain set in controlled crosses (Hwang *et al*., 1991). Duration of stigma receptivity affected the grain set in controlled crosses. It is well known that there is a variation among plant species for the success of hybridization. This may due to the variation occur in duration of pollen viability, duration of stigma receptivity and optimum time of pollination. Page and Stucker (1990) studied the factors affecting success of controlled crosses of wild rice (*Zizania palustris* L.). They suggested that the traditional pollination procedures described by Elliot (1980) had resulted in recurring problems with low grain

set in Minnesota wild rice breeding program which hindered progress in breeding studies. The aim of this study is to find out the factors which may affect success of controlled crosses in rice cv. Agamy hopping to develop a pollination procedure that would result in increased grain set.

MATERIALS AND METHODS

Grains of rice *Oryza sativa* L. cv. Agamy were secured through the traditional farmers of Sersna village 5 Km east of EL-Fayum City, Egypt. Page and Stucker (1990) described the procedures followed in this trail with little modification to meet local circumstances and available facilities. In greenhouse, grains were germinated and transplanted 30 days old to 20 cm. pots containing loamy soil supplemented with super phosphate, ammonium nitrate and zinc sulphate. The greenhouse was not environmentally conditioned as it was covered with theran (20% shaded). All recommended field practices were followed. The experimental work was conducted through the summer seasons of 1999 and 2000. The trial was conducted twice in the same season. So, data were collected on two groups of plants at five days intervals during the same season. Plants used as male were arranged in the southern corner of the greenhouse to avoid air movement and pollen dispersion. Plants used as female were arranged in the northern corner of the greenhouse. Female plants were emasculated daily to avoid any uncontrolled pollen shed. The experiment layout was randomized complete design with three replicates. The following aspects were studied: 1)- Duration of pollen viability. Where, the time of extrusion of anther from the florets is used to determine the four pollen age groups that considered as treatments for pollen viability on 8 August 1999 and on 13 August 2000 during the first and second seasons respectively. The used four ages for studying pollen viability were immature, 0-2 h, 2-4 h or 12 h. (2)- Duration of stigma receptivity. In which 45 female plants were chosen at heading age. Flag leaf was removed and the first 15 apical spikelets of each panicle were used to get stigmas of age ranging between 0 to 7 days. In the same time, the other florets were removed from the panicles and the rest of panicles on the plant were also removed. During the seven days plants were transferred regularly to the male area in the greenhouse early in the morning and plotted with pollination treatment. The percent of grain set was determined after three weeks from pollination. 3)- Effect of hour of pollination. Emasculated panicles of female plants were pollinated at one-hour intervals starting from 6.00 AM. to 3.00 PM. So, ten entries were considered as treatments for effect of hour of pollination on grain set. Plants after pollination were covered with 15X35 cm. paper craft bags. This experiment was repeated 5 days later on the other group in both seasons.

The grain set percentages were converted to its corresponding cosign values (Angle) and subjected to conventional statistical analysis methods according to computer software ‘MSTAT’ (1990) designed for statistical analysis. Where, factorial analysis of variance and combined analysis of variance over seasons were computed then mean separation was done according to Duncan's multiple range at 5% level of significance.

RESULTS

1- Effect of pollen age

Data in Table (1) represent the grain set percentages of the four chosen pollen ages over the two experimental groups during the two seasons. The analysis of variance revealed that pollen age resulted in significant differences on the percentages of grain set. While, no significant differences were realized between seasons. In both seasons, it was clear that 0-2 h pollen age resulted in the greater grain set percentage 41.0 and 44.6% for the first and second seasons, respectively. However, the immature pollen age showed a significant reduction in grain set percentage as compared with 0-4h pollen age.

Data strongly proved that pollen viability was greatly decreased as the pollen age increased. Though, 6 and 12-hour pollen ages showed a considerable reduction in grain set percentages. This reduction reached its maximum at 12-hour pollen age as the corresponding grain set percentages were ranged between 0.9 and 1.17% for the first and second season, respectively.

Table 1: Mean percent grain set of panicles pollinated with pollens of different ages in rice cv. Agamy during two successive seasons 1999 and 2000

Season / Pollen Age	First Season			Second Season			Combined 1999/2000
	First group	Second group	Average 1999	First group	Second group	Average 2000	
Immature	18.2	7.7	12.95 B	12.2	9.8	11.00 B	11.98 b
0-2 h	35.9	46.2	41.05 A	53.1	36.1	44.60 A	42.82 a
2-4 h	6.8	4.6	5.70 C	20.1	7.4	13.76 C	9.73 c
4-6 h	4.2	5.1	4.62 D	11.4	4.2	7.13 D	5.43 d
12 h	0.0	1.8	0.90 D	2.3	0.0	1.17 D	1.03 d

Source of variance	Degree of freedom	Mean squares
Seasons	(a-1) 1	9.1 ns
Groups	(b-1) 1	169.8 *
Pollin age	(c-1) 3	943.1 *
Season X Group	(a-1) (b-1) 1	1035.1 *
Group X Poll. Age	(b-1) (c-1) 3	13.4 ns
Season X Poll. Age	(a-1) (c-1) 3	79.1 ns
Se X Gr X X Poll.	(a-1) (b-1) (c-1) 3	2156.1 *

Means have the same letter(s) are not significantly differed at 5% level

The same trend could be noticed from the combined analysis over the two experimental seasons. There was a significant interaction between pollen age, groups and seasons. This may be due to the highly significant effect of pollen age as the values of treatment mean squares were considerably high.

So, it could be stated that the pollens over two hours old resulted in a low grain set percentage and its viability was greatly affected by the time elapsed since its excursion from the florets. It is clear from Figure (1) that pollen grains aged 4-6 and 12 hors were smaller in size and many deformations was observed which may affects its viability.

fig1

2- Effect of duration of stigma receptivity

As for the duration of stigma receptivity, it was noticed from analysis of variance that stigma age had significant effect on the percentages of grain set. While no significant effects were realized due to both seasons and groups. The combined data over the two seasons presented in Table (2) revealed that, grain set percentages were significantly different according to the stigma ages. Where, stigmas of 1 or 2 days old resulted in the highest grain set percentage.

The corresponding grain set percentages were 17.42 and 24.08% for the first and the second age, respectively. A rapid reduction was occurred in grain set percentage when the age of stigmas more than three days (figure, 2). Where 50 to 80% reduction in grain set was realized. Stigmas with age over 5 days showed a comparable low grain set percentages. No significant differences could be found between stigmas aged six and seven days, indicating that stigma receptivity was greatly affected by its age (the time elapsed since its emergence from the florets) and the proper age for stigmas to receive the pollen was one to two days old.

Table (2): Grain set percentage resulted from panicles with different stigmas age (1-7 days) in rice cv. Agamy during two successive seasons 1999 and 2000

Grain Set percentage							
Days	First season			Second season			Combined 1999/2000
	First group	Second grop	Average	First group	Second grop	Average	
1	14.57	22.03	18.3 B	19.40	13.67	16.5 B	17.420 b
2	22.80	22.77	22.0 A	27.57	23.17	25.4 A	24.080 a
3	10.97	10.27	10.6 C	13.33	12.60	8.3 C	11.790 c
4	7.60	11.10	9.4 C	9.73	6.93	8.3 D	8.842 d
5	4.70	7.06	7.0 D	8.43	7.23	7.8 D	7.400 d
6	4.70	7.06	5.9 D	3.96	3.56	3.8 E	4.842 e
7	1.63	3.13	2.4 E	1.73	2.06	1.9 E	2.142 f

Source of variance	Degree of freedom		Mean squres
Seasons	(a-1)	1	6.4 ns
Groups	(b-1)	1	13.5 ns
Receptivity	(c-1)	3	315.2 *
Season X Group	(a-1) (b-1)	1	24.5 ns
Group XRecept.	(b-1) (c-1)	3	44.2 ns
Season XRecept.	(a-1) (c-1)	3	215.3 *
Se X Gr X XRec	(a-1) (b-1) (c-1)	3	426.9 *

Means having the same letter(s) are not significantly differed at 5% level

3- Effect of time of pollination on grain set

Data presented in Table (3) for the mean percent grain set over the two individual season or the combined two seasons of the ten chosen hours for pollination reveal a significant response on the percentages of grain set due to the changes in time of pollination. Generally the mean percent of the grain set was considerably low for all chosen times of pollination in the two seasons.

fig2,3

No significant effects were found due to season or group. The period ranging between early morning (6 Am.) to mid morning (9 Am.) showed the high mean percent of grain set. Where, grain set percentages ranged between 20.75 to 18.38% and no significant differences could be recognized among those hours. Pollination time after 10 am greatly resulted in a decrease in mean percent of grain set. This reduction reached its maximum at noon or later on. The constant reduction occurred in the mean percent of grain set at none was realized in both experimental groups and seasons. So, data suggest that the proper time for pollination ranged between six to nine in the early morning.

Table (3): Mean percent grain set of panicles pollinated at one-hour intervals from 6 AM. to 3 PM. of rice cv. Agamy during two successive seasons, 1999 and 2000.

Grain Set percentage							
Time	First season			Second season			Combined 1999/2000
	First group	Second grop	Average	First group	Second grop	Average	
6 AM	18.7	24.0	21.35 A	18.0	19.3	18.62 A	20.00 a
7 AM	20.3	22.7	21.50 A	18.0	19.3	18.65 A	20.75 a
8 AM	19.0	18.3	18.65 A	16.3	20.3	18.30 A	18.38 a
9 AM	16.3	19.3	17.80 A	17.6	12.8	15.20 B	16.50 a
10 AM	14.0	15.3	14.65 B	16.0	12.0	14.00 B	14.33 b
11 AM	9.4	8.0	8.70 C	8.0	4.0	6.00 C	7.35 c
12 AM	5.3	11.7	8.50 C	8.7	6.3	7.50 C	8.00 c
1 PM	6.7	8.7	7.70 D	4.7	6.7	5.70 C	6.70 c
2 PM	10.7	5.3	8.00 C	6.3	5.3	5.80 C	6.90 c
3 PM	4.7		7.50 C	6.7	9.1	7.90 C	7.70 c

Source of variance	Degree of freedom		Mean squares
Seasons	(a-1)	1	18.2 ns
Groups	(b-1)	1	13.5 ns
Pollination hour	(c-1)	3	399.2 *
Season X Group	(a-1) (b-1)	1	11.6 ns
Group X Poll.	(b-1) (c-1)	3	44.2 ns
Season X Poll.	(a-1) (c-1)	3	58.3 ns
Se X Gr X X Poll.	(a-1) (b-1) (c-1)	3	37.7 ns

Means having the same letter(s) are not significantly differed at 5% level

DISCUSSION

Pollination takes place when pollen is most abundant, but time of day of pollination is generally not as important as pollen quality (Hallauer and Sears 1966; Brown and Shands 1956). The results of this trail indicate that in rice cv. Agami, the pollens over two hour old resulted in a low grain set percentage and their viability is greatly affected by the time elapsed since their exctrusion from the florets. Rice pollen was only viable for a short period of time, with an approximately 50% loss of viability within 20 min. of shedding (Khatun and Flowers, 1995). In many other self-pollinated crops the pollen

viability is limited by a very short period to be viable. Pollens of wheat have been shown to remain viable for only 15 min Brown and Shands (1956) and D'Souza (1972). In barley the longevity of pollen and stigmas was studied and the pollen viability was 3-4 hours as mentioned by Anthony and Harlan (1920). The low grain set obtained in wild rice cross pollination in green house was attributed to the practice of collecting pollen from florets that had opened overnight. This probably resulted in a large portion of the collected pollen being non-viable Page and Stucker (1990). In Egypt it is used to collect pollens directly from the male parent just before crossing and this allows collecting pollens from florets that opened overnight and thus the pollen age is more than 10 hours. Using the immature pollens (from closed florets) also gives low percent of grain set and the collected pollens appear to be more sticky to be separated over the female panicle. The results of this study suggest that to insure high grain set percentages in controlled crosses of rice cv. Agamy the pollens must be collected within two hours from extrusion of anthers.

It is worthy to mention that, Ananthakalaiselvi *et al*.,(1999) reported that fresh and 1 h stored pollen were useful for good grain setting. The stigma was receptive up to 2 days of starvation (70.7% grain set).

Regards the rice cv. Agamy stigmas showed long period of receptivity after emergence from the floret (about five days). This factor may not much interrupt the percentage of grain set in controlled rice cv. Arabi pollination but, gives flexibility to retain successful pollination. In many self-pollinated and cross-pollinated crops the stigma receptivity remains for several days. Experimental evidence suggests that exerted stigma traits, in particular its receptivity (probability of fertilization and grain set on spikelet with exerted stigma by artificial pollination after flowering) in rice would increase outcrossing rates Xu and Shen, (1988).

Data of the time of pollination suggested that the proper time for pollination ranged between six to nine AM. A considerable decrease in mean percent of grain set was found when controlled cross was applied after 10 AM. So, the success of controlled cross is affeted by the time of day the pollination is made. This disagrees with the finding given by Page and Stucker (1990) who suggested that pollination time did not influence the controlled cross in wild rice. The distinction in the fore mentioned findings might be attributed to the changes in temperature and relative humidity during the day inside the greenhouse where the trial was done. Many environmental factors affect the proper grain set in rice plants. To ensure pollination success and spikelet fertility, responses of upland rice genotypes to a water deficit at anthesis could be evaluated using anther dehiscence, pollen shedding and pollen germination in controlled conditions Hallauer and Sears (1966) and Ekanayake *et al*. (1990).

CONCLUSION

To conclude, the Egyptian technique of crossing used in rice leads to low grain set percentage. It is suggested to conduct the following steps in order to achieve high grain set during hybridization of rice cv. Agamy:

A- Clip all male florets with exposed anthers from the pollen parent.
B- Pollen collection should take place within 2 h after the old florets are clipped from the male plant (don't store pollens more than 4 hours).
C- Care must be taken not to dislodge the pollen from the anthers during collection.
D- Agamy cultivar stigmas showed long period of receptivity after emergence from the floret (about five days).
E- Pollination must be done between 6 to 9 Am. early in the morning.
F- Using a forceps to shake the collected florets above the female panicle does pollination.

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دراسات على بعض العوامل المؤثرة على نسبة عقد الحبوب الهجينية المتحكم فيها في نبات الأرز

على محمود صبـــور

قسم النبات الزراعي – كلية الزراعة – جامعة القاهرة

تم أجراء هذا البحث على أحد الأصناف المحلية من الأرز (صنف عجمي) خلال موسمي ١٩٩٩ و ٢٠٠٠ بغرض التعرف على بعض العوامل التي تؤثر على نسبة عقد الحبوب أثناء التحكم في التلقيح لأنتاج الهجن في نبات الأرز. و العوامل تحت الدراسة هي: عمر حبة اللقاح – فترة حيوية المياسم – وقت أجراء التلقيح و كانت أهم النتائج:-

١- تؤثر حبوب اللقاح ذات عمر أكثر من ساعتين بدرجة ملحوظة على النسبة المئوية لعقد الحبوب.

٢- تنخفض حيوية حبوب اللقاح بدرجة كبيرة مع مرور الوقت منذ انتثارها من المتك و تصبح قريبة من الصفر بعد مرور ١٠-١٢ ساعة من انتثارها.

٣- كانت أعلى نسبة مئوية لعقد الحبوب مع المياسم من ١-٢ يوم

٤- تنخفض قدرة المياسم على استقبال حبوب اللقاح بمرور الوقت و تصبح قريبة من الصفر عند عمر ٦-٧ أيام.

٥- حقق أجراء التهجين من الساعة ٦-٩ صباحا أعلى نسبة مئوية لعقد الحبوب.

٦- كانت النسبة المئوية لعقد الحبوب قريبة من الصفر عند أجراء التهجين خلال الفترة من الساعة صباحا حتى الساعة ٣ بعد الظهيرة.