

532

EVALUATION OF FACTORS AFFECTING SUCCESS OF CONTROLLED CROSSES OF WILD RICE (*Zizania palustris* L.) IN THE GREENHOUSE

N. J. PAGE and R. E. STUCKER

Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA. Contribution no 17312, the Minnesota Agricultural Experiment Station. received 30 Aug. 1989, accepted 26 Feb. 1990.

PAGE, N. J. AND STUCKER, R. E. 1990. An evaluation of factors affecting success of controlled crosses of wild rice (*Zizania palustris* L.) in the greenhouse. *Can. J. Plant Sci.* 70: 677-681.

The effects of pollen age, stigma age, and time of pollination were evaluated in wild rice (*Zizania palustris* L.) to develop a pollination procedure that would result in increased seed set in the greenhouse. Pollen age groups, ten stigma ages, and ten pollination times were investigated independently. Pollinations were done in the greenhouse during the months of November through April in 1986-1987 and 1987-1988. Percent seed set was used to assess the treatments. Pollen remained viable for only a short period of time. Maximum percent seed set was attained when pollen from anthers that had been extruded for less than 2 h was used in pollinations. Stigma receptivity remained consistent for 5 d then decreased rapidly. Time of pollination did not have an effect on percent seed set.

Key words: Wild rice, pollination biology, pollen viability, stigma receptivity, seed set

[Évaluation des facteurs influant sur la réussite des croisements contrôlés de riz sauvage (*Zizania palustris* L.) en serre.]

Titre abrégé: Biologie de la pollinisation du riz sauvage.

Nous avons évalué les effets de l'âge du pollen, de l'âge des stigmates et de la période de pollinisation du riz sauvage (*Zizania palustris* L.), dans le but de mettre au point une technique de pollinisation susceptible d'accroître la production de semences en serre. Les différents groupes de pollen, dix groupes de stigmates d'âge différent ainsi que dix périodes de pollinisation ont été étudiés séparément. La pollinisation a été effectuée en serre, de novembre à avril, en 1986-1987 et en 1987-1988. Les divers traitements ont été évalués en fonction de la production de semences. Comme la durée de viabilité du pollen est courte, les meilleurs résultats ont été obtenus lorsque la pollinisation était pratiquée dans les deux heures suivant l'extraction du pollen des anthères. La réceptivité des stigmates est demeurée constante pendant cinq jours, puis elle a diminué rapidement. La période de pollinisation n'a eu aucun effet sur les résultats.

Mots clés: Riz sauvage, biologie de la pollinisation, viabilité du pollen, réceptivité des stigmates, production de semences

Variation occurs among species for duration of pollen viability, duration of stigma receptivity, and optimum time of pollination. The effect that these traits have on seed set of controlled crosses has been adequately researched and defined for many crops. This is not the case with wild rice. Pollination procedures described by Elliott (1980) have resulted in

recurring problems with low seed set in the Minnesota wild rice breeding program. This has hindered progress in genetic and breeding studies.

In wild rice, very little pollen is shed until the plant is disturbed. Under natural or paddy conditions a slight breeze is all that is necessary to dislodge pollen from anthers. However, under greenhouse conditions where controlled crosses are made, anthers that have

emerged from male florets will not dehisce until the plant is manually agitated. This facilitates collection of large amounts of pollen to use in controlled crosses in the greenhouse. Prior to this research our standard pollen collection technique has been to bag panicles and leave them undisturbed until the following day when many anthers have been extruded. The pollen, collected by agitating the bag, was sprinkled on the stigmas of the female plants. This procedure has consistently resulted in low seed set. With the goal of developing reliable pollination procedures for wild rice in the greenhouse the objectives of this study were to determine the duration of pollen viability and stigma receptivity, and to examine the effect that time of pollination has on seed set.

MATERIALS AND METHODS

Plants were grown in the greenhouse during the winter months of 1986–1987–1988 using cultural practices described by Elliott (1980). Seeds were germinated in plastic dish pans filled with water and the seedlings were transplanted into 15-cm pots containing sterilized soil covered by approximately 0.5 cm of washed sand. The greenhouse soil mixture contained six parts field soil (Waukegan silt loam), six parts sand, five parts peat, and two parts manure. This resulted in a loamy sand texture. The water level was maintained 2–4 cm above the soil surface. The soil was supplemented with iron chelate while other nutrients were added as required. The temperature was maintained between 25 and 30°C and daylength was set at 16 h.

The greenhouse was partitioned with plastic sheeting to reduce air movement and thus prevent the spread of pollen between the male and female plants. Plants to be used as males were grown in the greenhouse on the side adjacent to the ventilation fans so free floating pollen would be pulled outside and away from the female plants when the fans were running. The plants to be used as females were emasculated daily to avoid any pollen shed, and to avoid having to bag panicles to prevent contamination.

Duration of Pollen Viability

Six pollen age groups: (a) immature, (b) 0–2, (c) 2–4, (d) 4–8, (e) 8–24, and (f) 24–32 h old were established as treatments to be evaluated. The group classification was based on time of extrusion

of the anthers from florets. For treatment (a), females were pollinated with pollen from unopened florets which were adjacent to florets that had extruded anthers. Anthers were extracted from the florets using a forceps to separate the lemma and palea. The pollen was extracted by rolling the anthers lightly between the thumb and forefinger. Pollen for the other five treatments was obtained from florets that had anthers extruded for specific time intervals. The pollen age intervals and isolation of pollen within the intervals are described below.

At 08:00 h the day before pollinations were made, all opened male florets were clipped from the male plants. At 17:00 h the same day, florets that had extruded their anthers since 08:00 h were isolated and identified with red tape. At 09:00 h the next morning, florets that had extruded their anthers since 17:00 h were isolated and tagged with blue tape. Florets that extruded anthers between 09:00 h and 13:00 h, 13:00 h and 15:00 h, and 15:00 h and 17:00 h were also isolated and marked. Florets were isolated by using iris surgical scissors to clip unopened florets from among florets with marked extruded anthers. At 17:00 h, florets with the six pollen ages were collected and the appropriate pollen was applied to the female plants. To dislodge and distribute the pollen from the anthers, the florets were shaken above the female panicle.

The experiment was set up as a randomized complete block design with three blocks. Blocks were attained through arrangement of plants into a two-by-three pot block in plastic lined boxes in the greenhouse floor space. Treatments were randomly assigned to female plants; thus, a female panicle was an experimental unit. Two runs of the experiment were completed in February, 1988 with 3 d between runs. Percent seed set was determined 2–3 wk after pollination by counting the number of developed seeds per panicle and dividing by the total number of female florets on the panicle. Duration of pollen viability was assessed using percent seed set from the six treatment means. Individual and combined ANOVA's were computed for the two runs.

Duration of Stigma Receptivity

Duration of stigma receptivity was determined by measuring seed set from pollinations of female plants manipulated to create a uniform stigma age on each panicle. Ten treatments, plants with stigmas ranging in age from 0 to 9 d, were obtained by clipping florets with emerged stigmas from the

m florets. For treatment (a), mated with pollen from unopened adjacent to florets that had anthers were extracted from the florets to separate the lemma and the lemma was extracted by rolling the floret between the thumb and forefinger. For treatment (b) a total of five treatments was obtained from the five anthers extruded for specific pollen age intervals and isolated from the intervals are described

day before pollinations were made. Male florets were clipped from the panicle at 17:00 h the same day, florets were isolated since 08:00 h were isolated with red tape. At 09:00 h the florets that had extruded their anthers were isolated and tagged with red tape. Anthers extruded between 09:00 h, 13:00 h and 15:00 h, and 17:00 h were also isolated and tagged. Pollen was isolated by using iris surgical blades to separate male florets from among florets and anthers. At 17:00 h, florets were collected and the pollen was applied to the female plants. The pollen from the male panicles were shaken above the female

panicle was set up as a randomized complete block design with three blocks. Blocks were arranged by arrangement of plants into a two-by-five-pot plastic lined boxes in the greenhouse. Treatments were randomly assigned to panicles; thus, a female panicle was the experimental unit. Two runs of the experiment were conducted in February, 1988 with 3 d intervals. Seed set was determined by counting the number of pollen grains per panicle and dividing by the number of florets on the panicle. Duration of pollen viability was assessed using percent seed set treatment means. Individual ANOVA's were computed for the

Receptivity

Receptivity was determined by comparing pollinations of female panicles to create a uniform stigma age range. For five treatments, plants were isolated from 0 to 9 d, were obtained from the emerged stigmas from the

panicle each day until at least 15 stigmas had emerged on a single day. Generally this occurred 2-3 d after initiation of stigma emergence on a panicle. On that day, the florets that did not have emerged stigmas were clipped from the panicle thereby leaving panicles with stigmas that had emerged from the florets over a 24-h period. The process was repeated at mid-morning over a 10-d period to establish the stigma age range of 0-9 d old.

The experiment was set up as a randomized complete block design with four blocks. Blocks were attained through arrangement of plants in a two-by-five-pot block. Individual panicles were used as experimental units. When the age ranges were established, the females were moved from the pollen-free area and females within each age group were assigned to blocks at random. Pollen from male plants was collected at mid-morning by holding a petri dish under undehisced anthers and tapping the stem of the plant to dehisce the pollen. Pollen was distributed on the females by sprinkling it from the petri dish onto the panicles. The first run of the experiment was completed in February 1987 and the second run in February 1988. Percent seed set was determined as described previously, and individual and combined ANOVA's were computed for the two runs to test for differences in stigma receptivity.

Effect of Hour of Pollination on Seed Set

Female plants were pollinated at 1-h intervals between 06:00 and 15:00 h; times of pollination formed the treatment variable. At the time of each pollination, a randomly chosen female plant was moved to a section of the greenhouse where no wild rice was growing. Florets with extruded anthers were collected from a randomly chosen male and immediately taken to the female for pollination. Pollination was achieved by shaking the anthers above the female panicles, after which the panicles were bagged to prevent contamination from pollinations to be done later in the day.

The experiment was set up as a randomized complete block design with day of pollination as the blocking factor; a female panicle comprised the experimental unit. One set of five male plants was used for blocks one and two while a different set was used for blocks three and four. A block was completed every other day to ensure adequate pollen production. Each male was used twice at random hour intervals, throughout the day. Three weeks after the last date of pollination, percent seed

set was assessed on the female plants and an analysis of variance was computed to determine if there were significant differences in seed set. The experiment was first conducted in November, 1986 and was repeated in April 1987.

RESULTS AND DISCUSSION

Duration of Pollen Viability

In the first run in February 1988, seed set averaged 42% when pollen from the 0- to 2-h pollen stage was used. No seed was set when pollen older than 2 h was used. In the second run of the experiment, seed set averaged 58% when pollen from the 0- to 2-h pollen stage was used. Seed set dropped to 15 and 6% respectively, when pollen of the 2- to 4-h stage and 4- to 8-h stage were used. Seed set obtained with pollinations using pollen of the 8- to 24-h stage showed an unexpected rebound to 39% (Table 1). Since pollen viability is not likely to increase once viability has decreased, the most likely explanation is that pollen from this pollen age group was contaminated with viable pollen that was younger than 2 hr. During the 16-h length of this stage, large clusters of male florets had opened, and unopened florets within the cluster could have been hidden from view. Hidden florets opening just prior to the time of pollen collection could have been a source of viable pollen that resulted in the rebound in seed set. We believe the "rebound" in seed set should be ignored and the conclusion drawn that pollen loses most of its viability after only 2 h of anther extrusion from the male floret. The combined analysis of variance showed that seed set effected from 0- to 2-h-old pollen was significantly higher than the other pollen age groups and that there was no significant interaction between pollen age and runs of the experiment.

Duration of Stigma Receptivity

Combined analysis of variance of the two runs of the experiment on duration of stigma receptivity showed there were no significant differences in seed set when stigmas of ages between 0 and 5 d were pollinated. There also was no significant interaction between stigma age and runs of the experiment. Seed set

Table 1. Mean percent seed set from pollinations using pollen from different age groups (immature to 24–32 h after anther extrusion) assessed in two experiments in February, 1988 under greenhouse conditions

Pollen age (h)	Percent seed set		
	Exp. 1	Exp. 2	Avg.
Immature	1.0 [†]	25.0	13.0
0–2	41.7	58.0	49.8
2–4	0.0	14.9	7.4
4–8	0.0	6.1	3.0
8–24	0.0	39.1	19.5
24–32	0.0	3.4	1.7
(SE) [‡]	4.4	9.8	5.4

[†]Each mean is based on three replicates with one panicle per replicate.

[‡]Standard error of means.

remained fairly stable for 5 and 6 days after stigma emergence in runs one and two, respectively, then dropped to near zero (Table 2). Previous to this experiment feathery, or newly emerged stigmas were believed to be receptive to pollen, while wilted, aged stigmas were expected to be unreceptive. These experiments showed that stigma appearance is not an accurate indicator of stigma receptivity. In both runs of the experiment, most stigmas remained feathery for 9 d or longer even though they were no longer receptive to pollen after 5 or 6 d.

Effect of Hour of Pollination on Seed Set

Both individual and combined analysis of variance of the two runs of this experiment showed no significant difference in seed set due to the different pollination times. Seed set was significantly higher in the April run than in the November run (potentially due to the use of females with nonreceptive older stigmas in the November 1986 run). However, the overall percent seed set for both runs was lower than the desirable rate. These results (Table 3) suggest that the success of controlled crosses in the greenhouse is not greatly influenced by the time of day the pollinations are made.

Wild rice seems to be similar to other crops regarding how time of pollination and duration of stigma receptivity affect seed set. For many crops, pollinations are made 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 research indicate that wild rice stigmas remain receptive for several days after emergence from the female floret. The duration should not be a factor limiting seed set in controlled pollinations, and it appears to be long enough to allow some flexibility in nicking of flowering between male and female parents.

Duration of pollen viability in wild rice appears to be atypical compared to other cross pollinating crops such as corn, pearl millet, and rye which have relatively long periods of pollen viability: 10 h or more in rye and corn and 1–2 d in pearl millet (D'Souza 1972; Jones and Newell 1948; Cooper and Burton 1965). The duration of pollen viability (approximately 2 h after anther extrusion) in wild rice resembles that of self-pollinated crops. Pollen of wheat and barley has been shown to remain viable for only 15 min to 1 h, and 3–4 h, respectively (D'Souza 1972; Anthony and Harlan 1920). This short pollen viability period in wild rice probably has played a major role in the limited seed set of past controlled crosses in the greenhouse. The previously used practice of collecting pollen from florets that had opened overnight, a practice similar to those used

Table 2. Mean percent seed set from pollination of panicles with different stigma ages (0–9 d) assessed in two experiments (February 1987 and 1988) in greenhouse conditions

Stigma age (d)	Percent seed set		
	Exp. 1	Exp. 2	Avg.
0	5.0 [†]	21.1	13.0
1	14.9	38.9	26.9
2	12.3	11.6	11.9
3	13.5	10.7	12.1
4	23.1	18.7	20.9
5	14.2	35.7	24.9
6	0.3	15.6	7.9
7	2.1	4.8	3.4
8	0.0	0.0	0.0
9	2.9	4.4	3.6
(SE)	7.4	6.5	4.9

[†]Each mean is based on four replicates and one panicle per replicate.

is generally not as important as viability (Hallauer and Sears 1966; Shands 1956).

Results of this research indicate that stigmas remain receptive for several days after emergence from the female floret. Time should not be a factor limiting controlled pollinations, and it is long enough to allow some flexibility of flowering between male parents.

Duration of pollen viability in wild rice is atypical compared to other cross-pollinated crops such as corn, pearl millet, and rye which have relatively long periods of viability; 10 h or more in rye (D'Souza and Newell 1948; Cooper and Harlan 1920). The duration of pollen viability in wild rice is only 2 h after anther extrusion, which resembles that of self-pollinated wheat and barley. Pollen viability of wheat and barley has been shown to remain viable for only 15 min to 3-4 h, respectively (D'Souza and Harlan 1920). This short duration of pollen viability probably plays a major role in the limited seed set in controlled crosses in the greenhouse. The previously used practice of opening anthers from florets that had opened in the greenhouse is similar to those used

Table 3. Mean percent seed set from pollination of panicles at 1-h intervals from 06:00 to 15:00 h assessed in two runs of the experiment (November 1986 and April 1987) in the greenhouse

Pollination time	Average percent seed set
06:00 h	11.7 ^{†‡}
07:00 h	19.8
08:00 h	17.3
09:00 h	14.3
10:00 h	14.6
11:00 h	9.4
12:00 h	11.2
13:00 h	20.9
14:00 h	19.4
15:00 h	9.8

[†]Each mean is based on eight replicates, four in each run.
[‡]Standard error of mean = 5.01%

with other cross-pollinated crops, probably resulting in a large portion of the collected pollen being non-viable and thus contributed to, or was the major cause of low seed set in wild rice cross pollinations.

The most critical step in attaining acceptable seed set is collection of viable pollen. Pollen collection procedures must isolate pollen sources and the pollen must be collected when it is most viable. The first step is to clip all male florets with exposed anthers from the pollen parent. Pollen collection should take place within 2 h after the old florets are clipped from the male plants. Care must be taken not to dislodge the pollen from the anthers during collection. The small amount of pollen available, because of only 2 h between isolation and collection of pollen,

prohibits the use of head bags to collect pollen because a large percentage would be lost. Pollinations are done by using a forceps to shake the collected florets above the female panicle. The anthers will dehisce and pollen can be observed as it falls on the panicle. The use of pollen from anthers that have been extruded for less than 2 h and the use of females with stigmas that have been extruded for fewer than 6 d should result in the highest percent seed set based on current knowledge of factors affecting seed set.

Anthony, S. and H. V. Harlan. 1920. Germination of barley pollen. *J. Agric. Res.* 18: 525-572.

Brown, B. M. and H. L. Shands. 1956. Factors influencing seed set of oat crosses. *Agron. J.* 48: 173-177.

Cooper, R. B. and G. W. Burton. 1965. Effect of pollen storage and hour of pollination on seed set in pearl millet, *Pennisetum typhoides*. *Crop Sci.* 5: 18-20.

D'Souza, L. 1972. A comparative study of the size and receptivity of the stigma in wheat, rye, *Triticale* and *Secalotricum*. *Z. Pflanzenzuchtg.* 68: 73-82.

Elliot, W. A. 1980. Wild Rice. Pages 721-731 in W. R. Fehr and H. H. Hadley eds. *Hybridization of crop plants*. American Society of Agronomy, Madison, Wis.

Hallauer, A. R. and J. H. Sears. 1966. Influence of time of day and silk treatment on seed set in maize. *Crop Sci.* 6: 216-218.

Jones, M. D. and L. C. Newell. 1948. Longevity of pollen and stigmas of grasses: buffalograss, *Buchloe dactyloides* (Nutt.) Engelm. and corn, *Zea mays* L. *J. Am. Soc. Agron.* 40: 195-204.

Table 4. Mean percent seed set from pollination of panicles at different stigma ages (0-9 d) assessed in two runs of the experiment (February 1987 and 1988) in greenhouse conditions

Exp. 1	Percent seed set	
	Exp. 2	Avg.
5.0 [†]	21.1	13.0
14.9	38.9	26.9
12.3	11.6	11.9
13.5	10.7	12.1
23.1	18.7	20.9
14.2	35.7	24.9
0.3	15.6	7.9
2.1	4.8	3.4
0.0	0.0	0.0
2.9	4.4	3.6
7.4	6.5	4.9

[†]Based on four replicates and one panicle