Pak. J. Bot., 7 (1): 33—40, 1975.

EFFECT OF POLLEN SIZE, POLLEN VIABILITY AND STIGMA RECEPTIVITY ON PERCENTAGE SEED SETTING IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)*

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Abstract

Percentage seed setting due to different time intervals after anthesis, of pollen size, viability and stigma receptivity was studied in variety HO1 of sunflower (*Helianthus annuus* L.).

One-way classification ANOVA revealed that the size of the pollen grains, their viability and stigma receptivity, at different time intervals after anthesis, had linearly negative effect upon percent seed set. This effect was found to be linearly negative. When the analysis of variance with regression was performed, and sum of squares due to regression and deviation from regression line partitioned, considerable amount of random heterogeniety was observed around the regression line. This was due to significant deviation of the treatments from the regression line.

With 23.38% or more reduction in size, the pollen viability was totally lost and seed setting adversely affected. Freshly viable pollen gave 94.44% seed set but their viability decreased with a proportional increase in their age. Similarly fresh stigma gave 93.33% seed set and with an increase in its age, receptivity proportionally decreased and affected the seed setting.

Introduction

A large number of flowering plants have mechanisms like heterostyly, protandry, protogyny and herkogamy that prevent self-pollination. Predominantly, they characterize the modification of a flower in such a way that makes cross pollination possible. Sunflower is also one of the numerous instances of these natural adaptations to cross pollination. The manner in which the rayflorets are developed on the capitulum and the nature of flowering of the discflorets, specially protandrous situation, are of interest to plant breeders as far as facilitation of cross pollination is concerned. The arrangement of the florets borne on the inflorescence determines the pollination control procedures.

The capacity of sun flower to set seed when selfed is quite low (Palowski, 1965) and it requires the help of insects rather than wind for cross pollination as the pollen grains are some what heavy and sticky. In addition to adaptations of attractive rayflorets and protandrous discflorets, other factors like temperature, humidity and the duration of pollen viability and stigma receptivity also affect pollination and ultimately seed set. Normal and conducive temperature conditions with optimum amount of relative humdity and sunlight are optimistically correlated with pollen viability and stigma receptivity which result in increased seed setting.

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*Part of the thesis submitted by senior author in partial fulfilment of the degree of M.Sc. Honours in Plant Breeding from the University of Sind, Jamshoro.

Pollen grains from freshly dehisced anthers when applied to emasculated stigmas, comparatively set more seed as compared to stored ones, for different intervals of time (Arnoldova 1926; Wagner, 1932 and Putt, 1940). However, different time intervals affect pollen viability and consequently seed setting, depending upon time and conditions of storage (Shareef & Khan, 1969, Poehlman 1959). Putt (1940) reported decrease in seed setting with increased age of sunflower stigma. The results showed that one, four, five and nine days old stigmas yielded 74.4, 63.0, 43.3 and 9.6% seed setting respectively. The author concluded that the emasculated flower should be pollinated within five days of emasculation to get the economic results. Shareef & Khan (1969) reported from their findings that even after twelve hours of emasculation, stigma showed maximum seed set but after 72 hours it lost its receptivity.

In the light of above information, investigations were undertaken to study the effect of pollen size, pollen viability and stigma receptivity at different time intervals after anthesis, on the percentage seed setting in an American commercial variety, HO1, of sunflower (*Helianthus annuus* L.). The choice of the crop was dictated by its leading position in the world production of edible oils. However, extensive use in margarine, shortening, paint, soap and potato-chip industry for domestic consumption has created interest for plant breeders and geneticsists towards the evolution of high yielding varieties. The implication of the present study aims at providing fundamental information regarding seed set production as a covariated response to optimum time of pollen viability and stigma receptivity.

Material and Methods

The experimental material consisted of a commercial variety of sunflower. The seed was sown on 28th February 1974 in an experimental area of uniform fertility with two seeds per hill at the distance of nine inches from hill to hill. Fifty rows were kept in total, each fifty feet long and at a distance of two feet between row to row. After two weeks of germination, the crop was thinned leaving single healthier plant per dibble.

Observations were recorded on pollen size, pollen viability and stigma receptivity. Pollen size was recorded in microns by measuring individual pollen grain diameter at fixed time intervals after anthesis through micrometry. Time intervals of 8, 12, 24, 36, 48, 60, 72, 84, 96 and 120 hours, respectively, were kept as treatments. Viability of the pollen grains was determined by acetocarmine reaction from each treatment. Those with pinkish red colour were considered viable even if reduced in size while those with no colour, irrespective of their size, were considered to be non-viable. This viability was then evaluated on the basis of percentage seed set from each time-interval treatment. Stigma receptivity was tested by emasculating disflorets and pollinating them with fresh as well as from the pollen from each time interval treatment. The florets were emasculated when the anther tubes had extended before bursting and bifurcation of stigma lobes. Remaining disc florets in the capitulum were totally removed. Similar time intervals, as in case of pollen size and viability, were maintained after emasculation. The emasculated florets from each treatment were pollinated by fresh pollen grains to assess stigma receptivity in terms of percentage seed set.

For statistical analysis, means were calculated for each treatment. One-way classification analysis of variance was performed for pollen size, pollen viability and stigma receptivity with respect to percentage seed set following the conventional procedure after Steel & Torrie (1960). Covariated response of individual treatment with respect to seed set was studied through regression analysis after Sokal & Rohlf (1969). The analysis of variance with regression was performed by keeping more than one value of dependent variable per value of independent variable (Snedecor & Cochran 1967) and by partitioning the sum of squares of deviation from the regression line.

Experimental Results

The average size of the pollen grains. their viability and stigma receptivity with respect to percentage seed set at different time intervals after anthesis are presented in Table 1. The one-way classification analysis of variance (ANOVA) of the complete data is given in Table 2.

Between treatment mean squares for pollen size are highly significant, which indicates that different time intervals after anthesis affect the size of the pollen grains. This kind of response is explained by regression coefficient value. The

TABLE 1. Pollen size, viability and stigma receptivity at different time intervals after anthesis and corresponding percent seed set values in HO1 variety of sunflower

Treatments in terms of time intervals in hours	Pollen size (microns)	% decrease in pollen size	% seed set by pollen of different ages	% decrease in pollen viability	% seed set by stigma recept-ivity	% decrease in stigma recept-ivity
Control	39.52	normal	94.44	normal	93.33	normal
8	37.48	5.16	74.44	21.17	90.00	3.56
12	37.12	6.07	63.33	32.94	88.88	4.76
24	34.01	13.94	56.66	40.00	67.77	27.38
36	33.75	14.60	34.44	63.53	43.33	53.57
48	33.86	14.32	20.00	78.82	33.33	64.28
60	33.04	16.39	11.11	88.23	11.11	88.09
72	32 86	16 85	6.66	92.94	7.77	91.67
84	32.08	18 82	2.22	97.64	—	100.00
96	30.28	23.38	—	100.00	—	100.00
120	28.95	26.74	—	100.00	—	100.00

TABLE 2. One-way classification ANOVA of pollen size, viability and stigma receptivity at different time intervals in sunflower varity HO1.

Character	Between treatments mean squares	Within treatments M.S. (Error)	Regression coefficient
D.F.	10	539	10
Pollen size	487.9374**	7.4015	—0.0756 ± 0.01**
Pollen viability	199.7584**	21.7661	—0.7824 ± 0.11**
Stigma receptivity	3228.3505**	30.1560	—0.5103 ± 0.18*

*Significant at 5% level.
**Significant at 1% level.

significant negative 'b' suggests a proportional decrease in the size of the pollen grains with a corresponding increase in their ages, after anthesis.

With respect to pollen viability, since the mean squares are highly significant, it may be concluded that different stages of viability of the pollen grains with respect to their ages, i.e., time interval after anthesis, do substantially exist which are reflected in terms of percentage seed set. This conclusion is supported by regression analysis where the time interval for pollen age is kept as independent variable and percentage seed set through pollen viability treated as dependent variable. The effect seems to be negative. Significant negative regression coefficient (b= —0.7824 ±0.119) shows that an increase in time interval or pollen age after anthesis proportionally decreases the seed setting.

As far as the effect of stigma receptivity after different time intervals over seed setting is concerned, the significant mean squares indicate substantial differences between the treatments. The regression coefficient was also negatively significant. This suggests that an increase in time interval after receptivity of the stigma causes proportional decrease in seed setting. Simultaneous analysis was carried out for pollen size, pollen viability and stigma receptivity as dependent variables per value of time interval as independent variable. The complete ANOVA is given in Table 3.

Significance of between-group mean-squares reveals substantial differences as far as three groups of pollen size, viability and stigma receptivity, with respect to seed setting, are concerned. This would mean that the amount of seed setting caused by the variation in the time intervals of stigma receptivity will be different as compared to the time intervals of pollen viability. Same kind of interpretation can also be applied to pollen size.

In order to explain this response in terms of cause-and-effect relationship, i.e., to determine the type of difference in seed setting percentage among three groups.

TABLE 3. Simultaneous ANOVA of three groups of pollen size, viability and stigma receptivity per value of time interval with respect to seed setting in sunflower variety HO1

Source of variation	D.F.	S.S.	M.S.	F value
Between treatments	10	60325.0251	6032.5025	15.9562**
Within treatments	22	8317.4125	387.0642	—
Total:	32	68642.4376	—	—

**Significant at 1% level.

a simultaneous regression ANOVA of pollen size, pollen viability and stigma receptivity was performed (first reading under each column of Table 4). The major quantities in this table are same as in single classification ANOVA of Table 3, but in addition, we have a sum of squares representing linear regression which is always based on one D.F. This sum of squares is subtracted from the S.S. among-groups, leaving the residual sum of squares (of 9 D.F. in this case) representing deviation from linear regression. The sum of squares due to linear regression represents the portion of S.S. among-groups explained by linear regression on time intervals. The S.S. due to deviation from the regression represents the residual variation scattered around the regression line. The S. S. within-groups is the measure of the items around each group mean. After testing the mean squares for regression we find it to be significant. This mens that linear regression of time interval has clearly removed a significant portion of variation of seed setting values. Thus our conclusions are that the pollen size, pollen viability and stigma receptivity are linear function of the time interval or age of the pollen and there is minimum amount of heterogeneity around the regression line because of the non-signifiance of means squares of deviation from regression.

TABLE 4. Regression ANOVA of pollen size, pollen viability and stigma receptivity with respect to percent seed set at different time intervals in sunflower Variety HO1

Source of variation	D.F.	S.S.	M.S.
Among	10	60325.0225	6032.5052**
groups	10	54922.7490	5492.2749**
Within groups (Error)	22	8317.4125	378.0642
(Error)	11	956.1343	86.9213
Line of regression	1	17646.2552	17646.2552**
		6621.1362	6621.1362*
Deviation from regression	9	1087.6799	120.8553
		19333.2798	2148.1422**

*Significant at 5% level.
**Significant at 1% level

In order to study the separated effect of pollen viability and stigma receptivity over percentage seed setting, a simultaneous regression analysis of only two dependent variables (pollen viability and stigma receptivity) per value of independent variable (time intervals) was carried out and the analysis of variance is given in the second reading of each column of Table 4. The results show that the groups differ significantly, i.e., individual group carries its characteristic effect upon seed setting. To test whether the differences among seed setting percentage values, due to pollen viability and stigma receptivity can be accounted for by linear regression line, mean squares of deviation from regression line were tested. Significance of mean squares for deviation from regression could mean either 'y' is a curvilinear function of 'x' or that there is a large amount of random heterogeneity around the regression line or the mixture of both the conditions may prevail. In the present case, since there are only two groups for 'x' variable, curvilinearity can be ruled out, and therefore, we suspect a certain amount of heterogeneity around the regression line.

Discussion

It has been concluded from Table 2 that pollen age of different time intervals affects the size of the pollen grains. This response was found to be negative through regression analysis. The negative kinetics of pollen size with respect to different time intervals explains an inverse proportional relationship of the dependent (pollen size) and independent (time intervals or pollen age) variables. The maximum decrease in size was obtained after 96 and 120 hours of anthesis (Table 1). This shows that an old age of pollen causes greater reduction in its size which makes it completely non-functional after 4 days. These results are graphically represented in Figure 1. A significant negative linear regression estimates a decrease of 0.0756 microns in pollen size with an increase of unit time interval. The deviations from the regression line in the graph are minimum and therefore it is not possible to explain the variability within treatments.

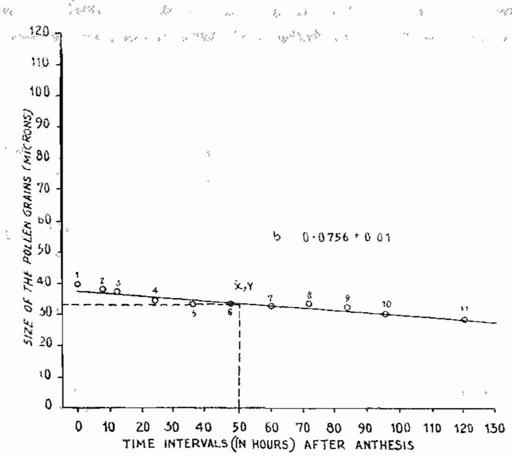

Fig. 1. Regression of time intervals on size of pollen grains in sunflower variety Ho I.

The effect of different time intervals on pollen viability in terms of percentage seed setting is analysed and the results were presented in Tables 2 and 4. The results indicated significant effect of pollen viability with respect to seed setting. The magnitude of variation explains proportional effect on seed setting (Figure 2). Significant negative regression line causes 78.24 percent decrease in seed setting with an increase of unit interval in pollen viability after anthesis. Treatments 6 and 11 bring drastic change in percent seed set and therefore would not be suitable for profitable results. The results are in agreement with those obtained by Shareef & Khan (1969) at Lyallpur, reporting that fresh sunflower pollen gave high seed setting and viability was reported to reduce with an advance of its age. The authors reported as high as 98.5% seed set with fresh pollen and viability lost beyond 108 hours after anthesis. The difference could be attributed to the varying environments of Tandojam and Lyallpur.

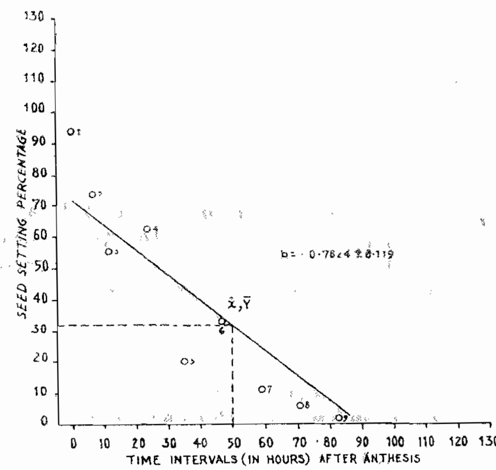

Fig. 2. Regression of time intervals on seed setting percentage of pollen viability in sunflower variety Ho 1

As far as the seed setting through stigma receptivity is concerned, the analysis of variance and regression coefficient is given in Tables 2 and 4. The variance ratio exhibited significant effect of different receptivity intervals over seed setting which was found to be proportionally negative through regression. The regression response is graphically represented in Figure 3. The regression coefficient shows that with an increase of twelve hour in stigma receptivity after emasculation, there is an average decrease of 51.03% seed setting. Treatments 2 and 3, that is, eight and twelve hours after receptivity, show maximum percentage of seed setting. Treatments 7 and 8 (60 and 72 hours, respectively, after stigma receptivity), with a mini-

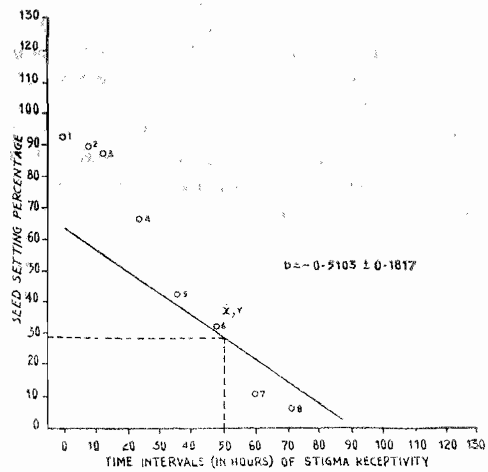

Fig. 3. Regression of time intervals on seed setting percentage of stigma receptivity in sunflower variety Ho 1.

mum seed setting percentage, possess maximum deviation from the regression line. This means that these particular time intervals are the most unstable or inconsistent with respect to seed setting. Treatments 5 and 6 are uniform in their performance because of their minimum deviation from the regression line.

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