Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS

Asian Journal of Plant Sciences

ISSN 1682-3974

DOI: 10.3923/ajps.2021.631.636

Research Article

Formation of Sunflower Seeds via Pollination Methodology Comparison Revealed by Self Incompatibility

Noer Rahmi Ardiarini, Darmawan Saptadi, Afifuddin Latif Adiredjo, Najla Thufailla Anessa and Umar

Department of Agronomy, Plant Breeding Laboratory, Faculty of Agriculture, Brawijaya University, Malang, East Java, Indonesia

Abstract

Background and Objective: Sunflowers have capitula that are hermaphrodite but protandry that causes self-incompatibility, which is an inability of plants to form seeds because the stamens occur before the pistil. This research aimed to understand the effect on the formation of sunflower seeds by comparing five pollination methods revealed by their self-incompatibility. **Materials and Methods:** The study had been conducted in Malang, East Java, Indonesia, from May until October, 2019 by using four genotypes, namely HA10, HA11, HA45, HA50 as the female parents and HA12 as the male parent. The genotypes were grown in field conditions. Several traits were observed after the genotypes being pollinated and the data were analyzed by analysis of variance (ANOVA). **Results:** The pollination method affected the flower head diameter, age of stamens and pistil occurrences, 100 seed weight, number of unfilled and filled seeds, the total number of seeds and seed set percentage but did not affect the flower head weight. On the other hand, genotype treatment affected the flower head weight, age of stamens and pistil occurrences, 100 seed weight and the number of filled seeds. Self-incompatibility criteria are strongly influenced by the successful seed formation that was obtained from the character of seed set percentage. **Conclusion:** The seed set percentages show that each pollination method's self-incompatibility criteria are high self-incompatibility for natural self-pollination, low self-incompatibility for manipulated self-pollination, cross-pollination, manipulated cross-pollination and very low self-incompatibility for open pollination. The four sunflower genotypes have the same criteria that are low self-incompatibility.

Key words: Criteria, incompatibility, pollination, seed, sunflower

Citation: Ardiarini, N.R., D. Saptadi, A.L. Adiredjo, N.T. Anessa and Umar, 2021. Formation of sunflower seeds via pollination methodology comparison revealed by self incompatibility. Asian J. Plant Sci., 20: 631-636.

Corresponding Author: Noer Rahmi Ardiarini , Department of Agronomy, Plant Breeding Laboratory, Faculty of Agriculture, Brawijaya University, Malang, East Java, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sunflower is a seasonal plant from the Asteraceae family, which was introduced from Mexico and Peru, Latin America. Not only as an ornamental plant but sunflower also has various benefits generated by the seeds. The seeds, the primary product of sunflowers are morphologically obtained from the disk florets. The seeds can be utilized for industrial purposes, including raw materials for food, medicine and cosmetics. Sunflower seeds can also be consumed as edible oil, which occupies the third-largest consumption in the world after soybean oil and palm oil[1]. Sunflower production problems have forced many industries in Indonesia to import sunflower seeds and oil due to a lack of domestic supply[2]. The low percentage of seed formation causes the lack of domestic supply. Sunflower has a capitulum which is hermaphrodite but protandry. Protandry is a condition where the pollen occurs before the pistil, which causes self-incompatibility.

Self-incompatibility is an inability of plants to form seeds because pollen or pistil heads are abnormal, for instance, the pollen tube grows too slow, hence damaging itself to fertilization or hindering it from reaching the surface of the pistil head[3]. Self-incompatibility is a form of infertility caused by a plant's inability that has normal pollen and pistil to form seeds due to physiological disorders that prevent fertilization[4]. Self-incompatibility can be caused by pollen not sticking to the pistil, pollen germinating on the stigma or pollen tubes fail to penetrate the stigma. Therefore, pollinating insects is very important to help the process of fertilization of the pistil by stamens and produce high-quality seeds[5].

Insects that act as the primary pollinators in sunflowers are bees and these insects have weather conditions that can stop their activity. Excessive hot or cold weather can slow down and even stop the bee colonies from looking for food. Bees activities usually occur at temperatures around 20-25°C and humidity from 65-75%[6].

This research aimed to determine the effect on sunflower seed formation by comparing five pollination methods revealed by its self-incompatibility, including natural self-pollination, manipulated self-pollination, cross-pollination, manipulated cross-pollination and open pollination.

MATERIALS AND METHODS

Description of sites and experimental designs: The research was conducted in Malang, East Java, Indonesia, from May-October, 2019. The materials consisted of four sunflower genotypes as female parents (HA10, HA11, HA45 and HA50) and one sunflower genotype as male parents (HA12), grown in field condition.

The pollination of sunflowers was achieved through five methods consisting of natural self-pollination, manipulated self-pollination, open pollination, cross-pollination and manipulated cross-pollination. Natural self-pollination covers sunflower heads with a plastic bag before flowering, making them pollinate naturally. Manipulated self-pollination is accomplished by covering sunflowers with a plastic bag before flowering but pollens are shed in a still-closed state to ensure self-pollination during the flowering phase. Open pollination is conducted by letting sunflowers naturally open themselves for pollination. Cross-pollination is completed by taking pollen from male parents and then placed on female parents. This pollination is repeated every day until the end of the flowering phase (the last stamen occurrence), then the flowers are covered with a plastic bag to avoid pollution by pollens from other plants. Manipulated cross-pollination is a similar procedure with cross-pollination but repeated only three times during the flowering phase.

The pollination method is an independent factor (P):

P1 : Natural self-pollination
P2 : Manipulated self-pollination
P3 : Open-pollination
P4 : Cross-pollination
P5 : Manipulated cross-pollination

Sunflower genotypes are a dependent factor (G):

G1 : HA10
G2 : HA11
G3 : HA45
G4 : HA50

The level of self-compatibility or incompatibility is calculated using the filled seed's ratio compared to total seeds (the number of filled and unfilled seeds) multiplied by one hundred percent[7]. The self-compatibility level (C) criteria are set as follows:

K : 0% = self-incompatibility
K : 1-24.9% = very low self-compatibility
K : 25-49.9% = low self-compatibility
K : 50-74.9% = high self-compatibility
K : 75-99.9% = very high self-compatibility
K : 100% = perfect self-compatibility

Statistical analysis: This research observed head diameter, head weight, the age of stamens and pistil occurrence, 100 seeds weight, number of unfilled (empty) and filled seeds, total number of seeds and seed set percentage. The obtained data analyzed using Analysis of Variance (ANOVA) with an F test at 5% level, then significant data is followed by the Least Significant Difference Test (LSD) at 5% level.

RESULTS

The pollination method affected the flower head (disk florets) diameter, age of stamen and pistil emergence, 100 seed weight, number of unfilled and filled seeds, the total number of seeds and seed set percentage but no effect on flower head weight in Table 1. On the other hand, genotypes treatment affected the flower head weight, age of stamen and pistil emergence, 100 seed weight in Table 2 and the number of filled seeds in Table 3.

The results of the variance analysis in Table 1 showed that the pollination treatment was significantly different in the character of head diameter, age of stamen emergence, age of pistil emergence, 100 seeds weight, number of unfilled seeds, number of filled seeds, the total number of seeds and seed set percentage. Treatment P1 produced sunflowers with the

Table 1: Variance analysis for all traits based on the pollination

Treatments	Characters observation							
	HD	HW	SE	PE	SW	US	FS	ST	SSP
P1	9.33^{a}	77.68	88.70^{b}	89.70^{b}	10.73^{c}	131.53^{b}	129.30^{a}	260.83^{a}	49.22^{a}
P2	9.08^{a}	72.08	81.48^{a}	82.48^{a}	7.68^{a}	78.53^{ab}	162.78^{a}	241.30^{a}	68.72^{c}
P3	10.48^{ab}	101.55	87.13^{b}	88.13^{b}	8.45^{a}	31.33^{a}	491.13^{c}	770.20^{b}	93.43^{d}
P4	11.33^{bc}	88.28	90.18^{b}	91.18^{b}	8.13^{a}	105.53^{b}	243.58^{b}	349.10^{a}	72.10^{c}
P5	12.56^{c}	103.30	88.33^{b}	89.33^{b}	9.77^{b}	209.87^{c}	296.35^{c}	428.80^{ab}	60.10^{b}
LSD 5%	1.43	ns	3.81	3.80	0.87	58.04	85.33	327.27	5.60

P1: Natural self-pollination, P2: Manipulated self-pollination, P3: Open pollination, P4: Cross-pollination, P5: Manipulated cross-pollination, HD: Head diameter, HW: Head weight, SE: Age of stamen emergence, PE: Age of pistil emergence, SW: 100 seeds weight, US: Number of unfilled seeds, FS: Number of filled seeds, ST: Total number of seeds, SSP: seed set percentage, data that has the same notation shows not significantly difference based on LSD 5%

Table 2: Variance analysis for several agronomical traits based on the genotypes

Treatments	Characters observation				
	HD	HW	SE	E	W
P1					
G1	9.20	84.50^{abc}	79.00^{de}	80.00^{de}	12.70^{g}
G2	9.20	84.50^{abc}	83.80^{e}	84.80^{e}	11.90^{fg}
G3	10.00	63.00^{ab}	113.20^{h}	114.20^{h}	6.30^{ab}
G4	8.90	78.70^{abc}	78.80^{de}	79.80^{de}	12.00^{fg}
P2					
G1	8.70	53.90^{ab}	80.20^{de}	81.20^{de}	8.00^{bc}
G2	7.90	65.80^{ab}	54.50^{a}	55.50^{a}	8.40^{cd}
G3	9.60	78.60^{abc}	113.00^{g}	114.00^{g}	6.10^{a}
G4	10.10	90.00^{abc}	78.20^{cde}	79.20^{cde}	8.20^{cd}
P3					
G1	9.00	64.40^{ab}	70.70^{bc}	71.70^{bc}	9.80^{de}
G2	9.30	72.10^{ab}	60.50^{a}	61.50^{a}	8.60^{cd}
G3	11.00	119.00^{cd}	105.00^{fg}	106.00^{fg}	5.80^{a}
G4	12.60	150.70^{d}	112.30^{gh}	113.30^{gh}	9.60^{cde}
P4					
G1	12.40	115.00^{cd}	82.10^{e}	83.10^{e}	9.40^{cde}
G2	11.00	79.60^{abc}	68.70^{b}	69.70^{b}	8.30^{cd}
G3	11.40	61.90^{ab}	101.80^{f}	102.80^{f}	5.90^{a}
G4	10.50	96.60^{bc}	108.10^{fgh}	109.10^{fgh}	8.90^{cde}
P5					
G1	12.40	88.60^{abc}	74.40^{bcd}	75.40^{bcd}	10.60^{ef}
G2	11.90	79.30^{abc}	69.40^{b}	70.40^{b}	9.40^{cde}
G3	9.90	47.30^{a}	102.60^{f}	103.60^{f}	6.00^{a}
G4	16.40	198.00^{e}	106.90^{fgh}	107.90^{fgh}	13.10^{g}
LSD 5%	ns	42.82	7.61	7.60	1.75

P1: Natural self-pollination, P2: Manipulated self-pollination, P3: Open pollination, P4: Cross-pollination, P5: Manipulated cross-pollination, G1: HA 10, G2: HA 11, G3: HA 45, G4: HA 50, HD: Head diameter, HW: Head weight, SE: Age of stamen emergence, PE: Age of pistil emergence, SW: 100 seeds weight, ns: Not significant, data that has the same notation shows not significantly difference based on LSD 5%

Table 3: Variance analysis for several seed characters based on the genotypes

	Characters observation			
Treatments	FS	US	ST	SSP
P1				
G1	108.90[ab]	115.60	224.50	48.53
G2	100.30[a]	108.80	209.10	48.05
G3	160.70[abc]	150.70	311.40	51.16
G4	147.30[abc]	151.00	298.30	49.16
P2				
G1	110.70[ab]	43.80	154.50	72.99
G2	169.30[abc]	82.70	252.00	67.08
G3	176.60[abc]	87.00	263.60	68.79
G4	194.50[abc]	100.60	295.10	66.05
P3				
G1	261.70[bc]	22.90	284.60	92.15
G2	228.20[abc]	19.20	247.40	92.36
G3	753.30[e]	30.00	783.30	96.34
G4	721.30[e]	53.20	774.50	92.89
P4				
G1	263.40[bc]	175.10	438.50	65.29
G2	248.60[abc]	84.70	333.30	73.24
G3	229.40[abc]	78.30	307.70	74.82
G4	232.90[abc]	84.00	316.90	75.04
P5				
G1	172.50[abc]	379.80	552.30	41.42
G2	179.50[abc]	134.40	313.90	58.19
G3	299.30[c]	103.80	403.10	74.43
G4	534.10[d]	221.50	661.90	66.37
LSD 5%	156.81	ns	ns	ns

P1: Natural self-pollination, P2: Manipulated self-pollination, P3: Open pollination, P4: Cross-pollination, P5: Manipulated cross-pollination, G1: HA 10, G2: HA 11, G3: HA 45, G4: HA 50, US: Number of unfilled seeds, FS: Number of filled seeds, ST: Total number of seeds, SSP: Seed set percentage, ns: Not significant, data that has the same notation shows not significantly difference based on LSD 5%

Table 4: Self-compatibility criteria

Pollination methods	Self-compatibility criteria
P1	Low
P2	High
P3	Very high
P4	High
P5	High
Genotypes	
G1	High
G2	High
G3	High
G4	High

P1: Natural self-pollination, P2: Manipulated self-pollination, P3: Open pollination, P4: Cross-pollination, P5: Manipulated cross-pollination, G1: HA 10, G2: HA 11, G3: HA 45, G4: HA 50

heaviest 100 seeds weight with an average of 10.73 g. The P2 treatment produced sunflowers with the fastest age of stamen and pistil emergence with a time mean of 81.48 and 82.48 days, respectively. The P3 treatment produced sunflowers with the highest number of filled seeds 491.13 and the highest total number of seeds 770.20 and the highest seed set percentage 93.43%. Treatment P5 produced sunflowers with the largest head diameter of 12.56 cm, the heaviest head weight of 103.3 g and the highest number of unfilled seeds 209.87.

The analysis of variance in Table 2 showed that the genotypes nested in the pollination method were significantly different in terms of head weight, age of stamen emergence, age of pistil emergence and 100 seeds weight. Sunflowers with the largest head diameter of 16.40 cm, heaviest head weight 198 g and 100 seeds weight 13.10 g were found in treatment G4 (P5). Sunflowers with the fastest age of stamen emergence 54.5 days and the fastest age of pistil emergence 55.5 days were found in the G2 (P1) treatment.

The variance analysis in Table 3 showed that the genotypes nested in the pollination method were significantly different in terms of the number of filled seeds. Sunflowers with the most number of filled seeds 753.30, the most number of seeds 783.30 and the highest percentage of seed set 96.34% were found in treatment P3 (G3). The sunflower with the highest number of unfilled seeds 379.80 was found in treatment P5 (G1).

The self-incompatibility criteria are strongly influenced by the character of seed set percentage-the higher the percentage, the lower the self-incompatibility level. It can be seen from Table 4 that the self-compatibility criteria for the pollination method in treatment P1 are low self-compatibility, treatment P2, P3 and P5 are high self-compatibility and treatment P4 is very high self-compatibility. The self-compatibility criteria for the genotype in treatment G1, G2, G3 and G4 are high self-compatibility.

DISCUSSION

Through this research, open pollination was the best method to obtain the highest seed set percentage (93.43%), while the lowest seed set percentage was found in natural self-pollination (49.22%). This result is in line with many research related to sunflower pollination, such as the report of Meeuse[8], which was explained that sunflowers with hermaphrodite flowers (flowers that have stamens and pistils) have a way of preventing self-fertilization by shedding pollen before or after a time when the stigma on the same plant accepts a situation known as dichogamy. Sunflowers are plants that can self-pollinate and cross-pollinate but sunflowers are more likely to cross-pollinate. Accordingly, if cross-pollination does not occur, a sunflower as a hermaphrodite plant can pollinate itself-even though this mechanism is inefficient with the rate of success at 2%. Desai *et al.*[9] explained that the percentage of cross-pollination on sunflowers could reach 17-62% depending on the pollinator insects activity but if there are no pollinator, the percentage of formation sunflower seeds only range from 15-20%.

Cross-pollination between small head diameter and large head diameter can produce offspring with the possibility of large head diameter. The combination of genotype sunflower crossing TS1 (small head diameter)xTR6023 (large head diameter) has flowers with large head diameters. Head diameter is an essential indicator of the seeds that are produced. The ideal head diameter ranges from 16-22 cm[10].

The open pollination method produced the highest flower head weight of 47.13 g. According to Altayeb and Siham[11], this treatment showed that the head weight is 121% heavier than self-treatment, which produces a head weight of 21.33 g. A large head diameter does not necessarily equal the heavier head weight as the weight is also determined by the number of produced seeds per head-more filled seeds, heavier the head weight and vice versa.

The sunflower genotype has a different age of stamens and pistil occurrence depending on its genetic plant and the environmental factors. Hazmy *et al.*[12] stated that the HA10 genotype needs 57 days to flower, then for HA45 genotype needs 99 days to flowering. According to Cho*et al.*[13], the timing of flowering is determined by endogenous genetic components as well as various environmental factors, such as day length, temperature and stress.

HA10 has the heaviest 100 seeds weight and HA45 has the lightest. That is consistent with the statement of Hazmy *et al.*[12] that the heaviest weight of 100 seeds per plant was found in the HA10 genotype at 12 g and the lightest weight of 100 seeds per plant was coming from HA45 at 4.11 g. Suprapto and Supanjani[7] explained that if the number of seeds formed is smaller, then the photosynthate is fully transferred to the formed (available) seeds so that the weight of 100 seeds is greater and the weight of seeds per capitula is also higher.

Natural self-pollination has the highest unfilled seeds because the plastic bag can increase the flower head's temperature and humidity so that it can damage the stamens. According to Al-Amery *et al.*[14], the high temperature during flowering and seed formation can damage the stamens and reduce the seed's fertility, which can cause a high percentage of empty seeds.

Open pollination can produce high-filled seeds due to the assistance of bees. Bees are the most significant insects in the sunflower pollination process. Unlike other insects that visit flowers only for their food, bees visit a lot of flowers to fulfill the needs of their colony[15]. Manipulated cross-pollination is the second pollination that can produce high-filled seeds. Manipulated cross-pollination is more suitable for sunflower heads with a small diameter because the intensity of pollination is low, only three times during the flowering phase. Cross-pollination is ideal for both small and big diameter of the sunflower head but it is better applied to big diameter head. A sunflower head with a big diameter needs more pollen, therefore, the sunflower seeds to be pollinated daily. Open pollination positively affects seed formation[4]. The average estimate of seeds formed ranges from 725.5-3840.9 seeds, which is 43% higher than the average seeds successfully formed in self-pollination. On the other hand, the average total number of seeds in the cross-pollination treatment is intermediate. Factors that influence the success of crossing include self-incompatibility, pollen intensity (number of stamens transferred) and weather during pollination. Cold temperatures can inhibit pollination and fertilization. Optimum environmental temperatures for sunflowers range from 25-28°C.

The highest percentage of seed set was 93.43% during open pollination treatment[16]. Plastic bags in self-pollination plants can cause loss of stamen viability or decrease receptive stigma level. The high temperature and humidity in the head can also cause a low ability to form seeds. Devasirvatham *et al.*[17] added that high temperatures during the flowering period resulted in a decrease in germinating stamen's ability because the water contained evaporated more quickly and the receptive level of stigma was reduced.

The seed sets percentage under open pollination treatment was the highest at 91.96%, which was very different from other pollination methods[12]. According to Suprapto and Supanjani[7], if the self-compatibility criteria is very low, then the sunflower is still very dependent on the pollinator's presence. The self-compatibility level in the treatment of genotypes is included in the high criteria, which means the self-incompatibility is low. The difference in the genetic makeup of each genotype is the cause of diversity. Diversity in plant appearances due to genetic makeup differences may occur even if the plants are the same type.

CONCLUSION

The self-incompatibility criteria are strongly influenced by the success of seed formation from the character of seed set percentage. The best pollination method treatment was open pollination with a seed set percentage was 93.43% and very low self-incompatibility, which applies to all genotypes in this research. This research is valuable for enabling sunflower breeding to improve the interesting trait, particularly using the open pollination method.

SIGNIFICANCE STATEMENT

This study discovers the pollination of sunflower by revealing the self-incompatibility of plants can be beneficial for plant breeders and agronomists to increase the seed set percentage in sunflower. Therefore, this study will help the researcher to uncover the critical areas of low production of sunflower seed that many researchers were not able to explore.

REFERENCES

1. Gandhi, S.D., A.F. Heesacker, C.A. Freeman, J. Argyris, K. Bradford and S.J. Knapp, 2005. The self-incompatibility locus (S) and quantitative trait loci for self-pollination and seed dormancy in sunflower. Theor. Appl. Genet., 111: 619-629.
2. Katja, D.G., 2012. Commercial sunflower oil quality and extracted sunflower oil (*Helianthus annuus* L.). Sci. Sci. J., 12: 59-64.
3. Watanabe, M., K. Suwabe and G. Suzuki, 2012. Molecular genetics, physiology and biology of self-incompatibility in Brassicaceae. Proc. Jpn. Acad., Ser. B, 88: 519-535.
4. Cholid, M., 2014. Optimization of the formation of sunflower (*Helianthus annuus* L.) seeds through the application of pollen-induced germination agents and pollinators. News Res. Dev. Ind. Plant, Vol. 20.
5. Silva, N.F. and D.R. Goring, 2001. Mechanisms of self-incompatibility in flowering plants. Cell. Mol. Life Sci., 58: 1988-2007.
6. Puškadija, Z., E. Štefanić, A. Mijić, Z. Zdunić, N. Paradžiković, T. Florijančić and A. Opačak, 2007. Influence of weather conditions on honey bee visits (*Apis mellifera Carnica*) during sunflower (*Helianthus annuus* L.) blooming period. Agri. Sci. Prof. Rev., 13: 230-233.
7. Suprapto and Supanjani, 2009. Genetic analysis of quantitative traits and self-compatibility of sunflower on Ultisol. AktaAgrosia J., 12: 89-97.
8. Meeuse, B.J.D., 2020. Pollination. Encyclopedia Britannica. Available from:https://www.britannica.com/science/pollination[Accessed 29 April 2021]
9. Desai, B.B., 2004. Seeds handbook: Biology, Production, Processing and Storage. 2nd Edn., Oxford University Press (OUP), New York Pages: 800.
10. Siddiqi, M.H., S. Ali, J. Bakht, A. Khan, S.A. Khan and N. Khan, 2012. Evaluation of sunflower lines and their crossing combinations for morphological characters, yield and oil contents. Pak. J. Bot., 44: 687-690.
11. Altayeb, O.A.E. and S.K.A. Nagi, 2015. Efficacy of honeybees (*Aapis mellifera*) on the production of sunflower (*Helianthus annus* L.) seeds in the Sudan. J. Exp. Biol. Agric. Sci., 3: 191-195.
12. Hazmy, Z.D., Ainnurrasjid and Damanhuri, 2017. Rejuvenation and morphological characterization of sunflower's germplasm (*Helianthus annuus* L.). Crop Prod. J., 5: 1133-1142.
13. Cho, L.H., J. Yoon and G. An, 2017. The control of flowering time by environmental factors. Plant J., 90: 708-719.
14. Al-Amery, M.M., J.H. Hamza and M.P. Fuller, 2011. Effect of boron foliar application on reproductive growth of sunflower (*Helianthus annuus* L.). Int. J. Agron., Vol. 2011. 10.1155/2011/230712.
15. Chambó, E.D., R.C. Garcia, N.T.E. de Oliveira and J.B. Duarte-Júnior, 2011. Honey bee visitation to sunflower: Effects on pollination and plant genotype. Sci. Agric., 68: 647-651.
16. Kumar, M. and R. Singh, 2003. Pollination efficiency of *Apis mellifera* in seed production of sunflower (*Helianthus annuus* L.). J. Entomol. Res., 27: 131-134.
17. Devasirvatham, V., P. Gaur, N. Mallikarjuna, R. Tokachichu, R. Trethowan and D. Tan, 2012. Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. Funct. Plant Biol., 39: 1009-1018.