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Assessment of Almond (*Prunus dulcis L.*) for Self-Compatibility and Stigma Receptivity Period Based on Fruit Set Index

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ABSTRACT

Regarding to the self-compatibility test the 6 genotypes under the study show low self-fertilization and categorized in self-incompatible group, in evaluating the best time for hand pollination, the emasculated genotypes in the same day show significantly difference in responding to the hand pollination in the days After emasculation which the highest fruit set percentage recorded in 3 days after emasculation and decreased in other days when considering the 0 day to 8 days after emasculation the study shows the importance and emphasis of choosing the overlapping varieties in an orchard and considering the self-incompatibility of genotypes and pollinator in reform and development of new almond orchard.

Keywords- Almond, Self-incompatibility, fertilization, almond emasculation, stigma, pollen.

I. INTRODUCTION

Almond (*Prunus amygdalus Batsch*) is one of the most important temperate trees fruits. Globally, the area under almond cultivation is over 2.07 million ha with an annual production of 3.18 million metric tons and 1.53 t/ha average productivity (FAO, 2019). Afghanistan is known worldwide to produce quality almonds since a long time. The area under its cultivation is about 22 thousand ha and produced 39 thousand tons of in shell nuts with a productivity level of 1.77 ton/ha (Afghanistan statistical year book 2020). Fertilization is essential for almond production (Cousin and El Maataoui, 1998). The crop is mainly self-incompatible and requires cross-pollination (Pimienta et al., 1983; Socias i Company et al., 2002).

Pollination defined as the transport of pollen from the anthers to the stigma. At present full differentiation must be established in almond between the self-incompatible cultivars (most of the traditional ones) and the self-compatible cultivars (most of them recent releases of the different breeding programmes) (Socias i Company, 2002; Socias i Company *et al*., 2009). The effective pollination period (EPP), first introduced by Williams in 1966, is one of the most important factors determining successful fertilization. EPP is determined by the longevity of the ovule minus the time lag between pollination and fertilization, providing that this value does not exceed the length of stigmatic receptivity. As reviewed by Sanzol and Herrero (2001), the EPP is limited by three main events during the reproductive process; stigma receptivity, pollen tube kinetics and ovule longevity. Stigma receptivity refers to the ability of the stigma to support germination of viable, compatible pollen. It has been implicated as a factor limiting the EPP and fruit set in kiwifruit (Gonzalez et al., 1995b), apricot (Egea and Burgos, 1992), pear (Sanzol et al., 2003b) and cherry (Guerrero-Prieto et al., 1985; Furukawa and Bukovac, 1989). A short life span of ovules is limiting to EPP in sweet and sour cherries (Postweiler et al., 1985; Cerovic and Ruzic, 1992) and apricot (Burgos and Egea, 1993). Abnormalities of the ovule or embryo sac

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development limit EPP in olive (Rallo et al., 1981), avocado (Tomer et al., 1976) and almond (Pimienta and Polito, 1982). Unlike other Prunus species where well-developed embryo sacs are present at anthesis, almond ovules are in the megasporemother-cell stage at flower opening, and complete embryo sac maturation 7–8 d after anthesis (Pimienta and Polito, 1983). Since embryo sac development is stimulated by the presence of compatible pollen tubes in the style and final elongation growth of the embryo sac is promoted by cross-pollination (Pimienta and Polito, 1983), ovule longevity in almond may be less limiting to EPP than in species attaining a mature embryo sac at anthesis.

DeGrandi-Hoffman et al. (1989) reported that yield in almond is determined by the number of flowers per tree and the effective pollination period (EPP) The concept of EPP was introduced by Williams (1965) to assess floral receptivity in apple, and was defined as the period during which pollination was effective at producing fruit. Details of stigma receptivity have been studied in only a limited number of species (Shivanna, 2003). Optimal receptivity is variable and can be from a few hours after flower opening as in teak (Tangmitcharoen and Owens, 1997), to a few days after anthesis as in oak (Kalinganire et al., 2000) and Silene alba (Young and Gravitz, 2002). The objectives of this study were to evaluate the self-compatibility of some almond by bagging them in a carton and investigate their stigma receptivity under defined developmental stages and to clarify the relationship between stigma morphology, pollen germination and tube growth. Such information would allow a greater understanding of the self-incompatibility and how factors affect the EPP in almond, and thereby provide information providing strategies to optimize pollination and increase fruit set in almond.

Objective 1: Testing the 6 genotypes of almond for self-compatibility.

Objective 2: Testing one of the almond genotypes for stigma receptivity period and standardizing almond pollination time overlapping and right time for hand pollination in breeding program.

II. MATERIAL AND METHOD

The research conducted during the almond blooming period on March 2022 in Haj Jafari private garden in Kabul, (Altitude 1966.71 m, Latitude 34º29'47.6"N, Longitude, 69º01'20.9"E) three branches were bagged on each 6 almond genotype in a carton paper in popcorn flower bud stage for evaluation of self-compatibility and 9 branches were tagged on one of the almond Genotype13129 to determine the length of the period of stigma receptivity of almond flowers based on fruit set percentage. One of the flower branches on each tree were counted to give the percentage of fruit set resulting from natural cross pollination. And the other branches were tagged and emasculated in popcorn stage as control (not hand pollinated) the remaining flowering branch were emasculated in the same date but hand pollinated in the difference days after emasculation for evaluating the fruit set percentage regarding to hand pollination after emasculation date till 8 days.

The genotype 13129 trees were interplant with pollinizing varieties and an adequate supply of honeybees was provided. Flowers on the remaining branches were emasculated on March 15, **2022,** when most were in the "popcorn" stage with the petals near full size, but not yet separated to open. During favorable weather, such flowers will open from one to three days (unpublished data). In the emasculation procedure, the petals, anther and most of the floral tube are removed, leaving the pistil exposed. Since pollinating insects are only attracted to the resplendent flower parts, the emasculated ones are safe from uncontrolled pollination." The emasculated flowers on one of the branches on each tree were used as controls (not pollinated). Flowers on the other branches were cross pollinated with genotype G13125 pollen which were overlapping regarding to their blooming time, after different intervals of time, as shown in the table 2. The pollen was obtained by removing the anthers from almond genotype G13125 flowers and holding them in the laboratory until they shed their pollen. The pollen was applied to the stigmas of the emasculated G13129 flowers with a smooth brush. Weather throughout the pollination and fruit setting period of the G13129 almond was ideal for the experiment. On March the minimum temperature was 5.04 Celsius with a maximum of 16.12 Celsius. The mild weather continued and there was no rain until end of March. Frosts have not occurred on March during the experiment the percentages of fruit set were based on final counts made on April 25**,** after the normal period of fruit drop.

III. RESULT

The highest rate of the fruit set in the bagged branches among 6 genotypes were recorded in genotypes 13128 and 13130 25.61 and 22.96% respectively (Table 1) in the remaining genotypes the fruit set are less than 11 % to nil and shows that the genotype under study was self-incompatible.

The emasculated buds that were hand pollinated March 18, three days after emasculation, gave significantly higher fruit set (42.00%) comparing to the other treatments understudy (Table 2). Since the flowers were emasculated from one to three days before they would have opened normally, March 17 could be considered the average date of flower opening. Cross pollination on March 18, three days after emasculation, was comparable, therefore, cross pollination one day after normal flower opening. Apparently, this was the time the pistil was most receptive. When cross pollination was delayed until March 21, 6 days after emasculation (comparable to cross pollination 3 days after normal flower opening), fruit set was significantly reduced. When

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flowers were not cross pollinated until seven or more days after emasculation (comparable to five or more days after flower opening), fruit set was practically nil. And also flowers cross pollinated the same day they were emasculated, 15 March**,** or one day later, March 16 (comparable to cross pollination two days and one day before the flowers would have opened), gave lower fruit sets than those pollinated march 18**.** This showed a lower receptivity of immature almond pistils to pollen germination and fertilization.

Table 1: Result of almond testing for self-compatibility

Genotypes	Average fruit set
G13125	0.00
G13126	0.00
G13127	2.90
G13128	25.61
G13129	10.88
G13130	22.96

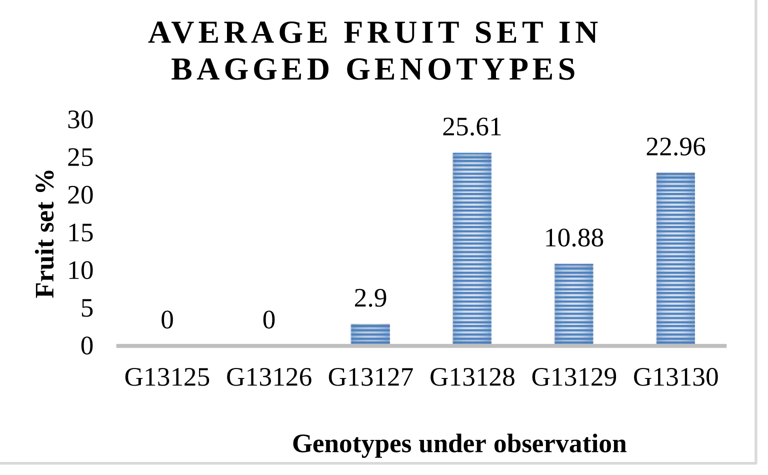
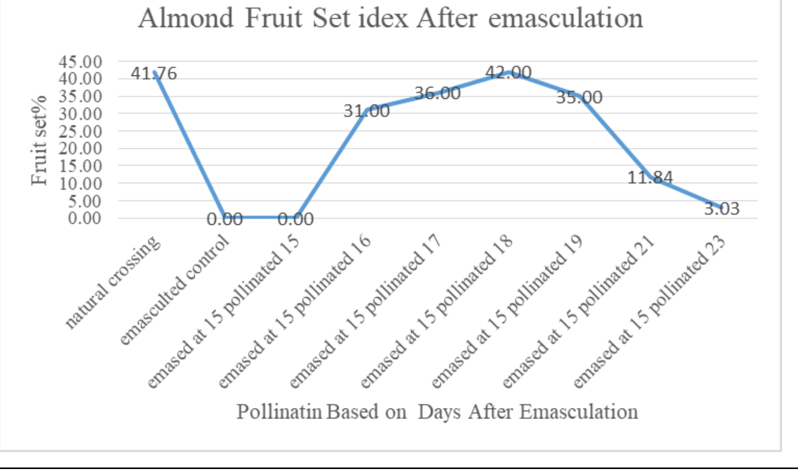


Table 2

Treatments	Days After Emas	Treatment	Date	Average fruit set	Temperature during the march: Average of Min	Temperature during the march: Average of Max	Wind speed km/h
Natural crossing		T1	12.3.2022	41.76			7.7
Emased control		T2	14.3.2022	0			7.4
Emased at March 15, pollinated 15	0	T3	15.3.2022	0			7.4
Emased at March15, pollinated 16	1	T4	16.3.2022	31			7.4
Emased at March 15, pollinated 17	2	T5	17.3.2022	36	5.05	16.13	9.3
Emased at March 15, pollinated 18	3	T6	18.3.2022	42			9.3
Emased at March 15, pollinated 19	4	T7	19.3.2022	35			33.9
Emased at March 15, pollinated 21	6	T8	21.3.2022	11.85			13
Emased at March 15, pollinated 23	8	T9	23.3.2022	3.04			11.1



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IV. DISCUSSION

Reviewing the angiosperm self-fertilization, Bertin(1993) conveyed that when more than 50% of pistil showed self-pollen tubes at the style base, the plant was considered to be self-compatible. When this percentage was higher than 75%, the plant was considered to be highly self-compatible. On the other hand, plants with <25% of the pistils with self-pollen tubes at the style base were considered to be self-incompatible, although in the most of self-incompatible plants<5% of pistils showed pollen tubes at their base. The plant with the percentage of pistils between 25% and 50% of pistils with self-pollinated tube at the style base were classified as of doubtful genotypes. Phenotypes were classified according to the mean number of self-pollen tubs observed at the style base. The range adopted were no pollen tubes, less than one pollen tubes, between one and three pollen tubes and more than three pollen tubes. In our case the highest self-fertilization rate shows 25.61% fruit set and all genotypes can be considered self-incompatible genotypes.

The results indicate that under weather conditions favorable for natural cross pollination, almond flowers are most receptive to effective cross pollination for a day or two days after they open, and remain receptive for only three or four days. The results also corroborate the conclusions of previous workers regarding the length of the period of receptivity. For example, tests with the apple have shown that pollination must occur within two to four days following the opening of a flower, if a high percentage is to set fruit. Previous studies at Davis have shown that any reduction in total number of almond flowers due to frost, disease or insect attack, caustic sprays, etc., or reduction in the proportion of flowers cross pollinated, causes a reduction in the final fruit set and yield. Although the almond normally produces many more flowers than develop into mature fruit, the loss of a portion of the flowers or the failure of a portion to be cross pollinated is not adequately compensated for by an increase in the set of-those remaining.

For maximum crops, therefore, essentially 100% of the flowers should be cross pollinated. Since a profitable almond crop depends upon cross pollination of practically all flowers, and each flower is receptive for only three or four days, it is essential that the grower maintain adequate combinations of cross-compatible varieties and provides an abundant supply of honeybees. The importance of strong colonies of honeybees for the almond orchard cannot be overemphasized since almonds bloom at a time when temperatures favorable for effective bee activity are generally restricted to one to three hours at midday.

The reduction of fruit set 4 d after pollination in 'Marcona' could be due to the loss of stigma receptivity, as reported in other studies (Kodad and Socias i Company, 2009; Ortega et al., 2004). The percentage of stigmas with pollen grains used to determine the stigma receptivity in our study showed a close fit with fruit set in both cultivars, indicating that EPP is limited by stigma receptivity and this is limited by temperatures at bloom. Burgos et al. (1991) also observed a rapid stigma degradation under high temperatures in apricot, with a subsequent shorter EPP.

The stigma is reported to be receptive at the time of anthesis in many tree crops such as peach, apricot, sweet cherry, apple and kiwi (Sanzol and Herrero, 2001). However, the receptive period can vary with species or cultivar, and requires delayed maturation of the stigma post-anthesis.

Ortega et al. (2004) pollinated almond flowers at 0, 2, 4 and 6 d after emasculation, and evaluated the number of pollen tubes in the style. Stigma receptivity varied with cultivar, and in some cases was optimal in youngest flowers and declined after 2 d; some cultivars were not receptive until 6 d after emasculation. Since flower stages at pollination were not defined, it is unclear if cultivars varied in flower development on their respective days after emasculation. Environmental conditions such as temperature at time of blooming may have impacted flower development and stigma receptivity. Vezvaei and Jackson (1995) achieved the highest fruit set in newly opened flowers. In contrast, Griggs and Iwakiri (1964) indicated that older staged flowers were effective at setting fruit; flowers pollinated 3 d after emasculation had higher fruit set than those pollinated at 0 or 1 d, and fruit set remained high for 7 d. Ortega et al. (2004) obtained acceptable fruit set following pollination from day 0 to day 4 after emasculation. In these studies, morphological descriptions of floral development were not given. Environment can play an important role in the rate of flower development, emphasizing the advantage of expressing receptivity on a developmental rather than a calendar basis.

V. CONCLUSIONS

The present study shows that the varietal association chosen by the farmers is not adequate in Afghanistan as recommended for almond. Thus, the selection of cultivars with the same flowering period than those that don't overlapping or cropping a single cultivar in farm is advised because it can be a means of improve almond yields in Afghanistan.

Our results show that the EPP, stigma receptivity and warm temperatures during bloom are possible influencing factors for fruit set and fruitfulness in Almond. These results emphasize the importance of early pollination to ensure an acceptable yield. Finally, the introduction and selection of adapted automatous cultivars and adequate honey bee population in orchard is advisable to increase and ensure almond production and for breeding program its important which act 2-3 days after emasculation.

ISSN: 2583-4053
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