COINCIDENCE OF FLOWERING TIME AND THE PRODUCTIVITY AND QUALITY OF CAULIFLOWER HYBRID SEEDS[1]

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ABSTRACT: The missing of flowering synchronization between the self-incompatible lines in a crop field of cauliflower hybrid seeds besides making the seed production smaller can compromise the genetic purity of them. The coincidence of the flowering time between two cauliflower lines was examined to study its effect on the productivity and quality of hybrid seeds. The treatments consisted of six different sowing dates, every fifteen days, using a self-incompatible tropical line pollinated by a winter line which does not present self-incompatibility. The following characteristics were evaluated: leaf average area and number of flowers per plant, number of siliques per plant, number and weight of seeds per plant, weight of thousand seeds and average number of seeds per silique. The germination standard test and genetic seed purity were determined for each treatment. The coincident flowering season between cauliflower lines affects directly the productivity and the genetic quality of the produced hybrid seeds. The closer the flowering time coincidence between the lines, the greater the number of seeds per silique and the smaller the percentage of non-hybrid seedlings. However, the coincidence of the flowering season between lines was found to influence physiological seed quality.
Key words: *Brassica oleracea,* seed, hybrid, self-incompatibility

COINCIDÊNCIA DE FLORESCIMENTO ENTRE LINHAGENS DE COUVE-FLOR NA PRODUTIVIDADE E QUALIDADE DE SEMENTES HÍBRIDAS

RESUMO: A falta de sincronismo de florescimento entre as linhagens auto incompatíveis em um campo de produção de sementes híbridas de couve flor pode além de reduzir a produção de sementes comprometer a pureza genética das mesmas. Com o objetivo de estudar o efeito da coincidência de florescimento entre linhagens de couve-flor na produtividade e qualidade de sementes híbridas, foi realizado o presente experimento. Os tratamentos consistiram em seis diferentes épocas de semeadura, espaçadas a cada quinze dias, de uma linhagem de verão auto-incompatível que foi polinizada por uma linhagem de inverno que não apresenta auto-incompatibilidade. Observou-se a coincidência do florescimento das diferentes épocas de semeadura com a linhagem polinizadora. Foram avaliadas as seguintes características: área foliar média, número de flores por planta, número de síliqüas por planta, número de sementes por planta (peso e número), peso médio de 1000 sementes e foi determinado o número de sementes por síliqüa. Foi realizado ainda, o teste padrão de germinação e determinada a pureza genética das sementes para cada tratamento. A coincidência da época de florescimento entre as linhagens de couve-flor afetou diretamente a produtividade e a qualidade genética das sementes híbridas produzidas, sendo que, quanto maior foi o nível de coincidência, maior foi o número de sementes formadas por síliqüa e menor a percentagem de sementes contaminantes. Entretanto, não teve influência na qualidade fisiológica das mesmas.
Palavras-chave: *Brassica oleracea,* semente, híbrido, auto-incompatibilidade

INTRODUCTION

The main mechanism of pollination control in *Brassica* is the sporophytic type self-compatibility system (Thompson, 1957). This phenomenon occurs when the ovule may not be fertilized by pollen grains of the same plant or another plant which carries the same alleles of incompatibility, causing the absence or drastic reduction of seed number per silique (Bateman, 1954; Watts, 1963; Thompson & Taylor, 1971). The understanding of such systems and its use has marked the appearance of the first commercial hybrids of this genus (Wallace & Nasrallah, 1968; Ikuta, 1974; Giordano, 1983; Silva, 1985; Riggs, 1988). It was demonstrated that homozygotic lines, type S1S1 and S2S2, planted in alternate lines in the field yield only hybrid seeds (Ramalho, 1990).

Takazaki (1984) observed that summer hybrids obtained from crossing between summer and winter are of intermediate precocity. However, these hybrids presented remarkable vigor, faster leaf development, and less sensitivity to buttoning than summer cultivars.

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Ikuta (1969) mentions that according to Professor Marcílio Dias data the generation F1 of cauliflower cultivars shows a remarkable hybrid vigor when inducing the crossing between the summer cauliflower cultivar, Piracicaba Precoce N°1, and the winter cultivar, Terezópolis. The resistance to heat and the precocity of the Piracicaba Precoce N°1 cultivar are dominant features. Consequently, the hybrids of this cultivar are vigorous, precocious and resistant to heat. The hybrids also show homeostasis, i.e., greater tolerance to environmental changes of temperature. This latter characteristic is very important, especially for plants grown in the surroundings of the São Paulo city. The temperature fluctuation in this city is harsh due to the influence of the near mountain range 'Serra do Mar'.

The variety Piracicaba Precoce Nº1 has shown self-incompatible plants since its early establishment which allowed the breeding with winter cultivars, and the production of hybrid seeds (Ikuta 1969). However, even working with self- incompatible varieties there is no guarantee about the genetic purity of the seeds. The use of lines carrying weak alleles for self-incompatibility and also plant interactions with the environment may cause the production of seeds in the mother line by self-fertilization or sib-crossing; giving rise to contaminant seeds. Furthermore, the need of sowing the summer lines out of season causes great reduction in seed productivity because there is no satisfactory plant development due to the induction of early flowering.

According to Maluf & Corte (1990), one of the main problems in cauliflower hybrid seed production is the absence of flowering coincidence in the parental lines. This fact would decrease the amount of seeds produced as well as their genetic purity. The use of lines with opposite cycles, like the summer and winter ones, may enhance the problem, since there is no way to foresee accurately when their flowering occurs. Therefore, if a perfect coincidence in flowering time is not found between the lines, the pollinator insects will work more in one of the lines doing a few movements among the plants of the two lines. Consequently, the number of contaminant seeds will increase. However, there are no published reports quantifying the importance of this sort of problem.

Arús at al. (1982) stated that many seed companies supervise the genetic purity of their hybrids before selling their seeds by sowing and checking the occurrence of atypical plants. However, this procedure requires a great deal of time, space and people support. The same authors observed values of contamination in different *Brassica oleracea* hybrid seeds as varying between 1.5 a 40.1%.

The present study aims to evaluate the effect of the coincidence of the flowering time between cauliflower lines on the productivity and quality of seeds.

MATERIAL AND METHODS

The study was carried out in Botucatu, SP, Brazil. The local have the following geographical characteristics: west longitude 48°26'37", south latitude 22°52'20" and an altitude of 800 m above sea level. The local climate Cfa type, with annual average temperature of 20,2°C and with average rainfall of 1,464 mm 0(1965-1993).

The production of cauliflower hybrid seeds from a summer self-incompatible line (S7) pollinated by a winter self-compatible line (S3). Was evaluated the S7 line came from the summer cultivar Piracicaba Precoce Nº1, while the other line S3 came from a winter line, obtained by crossing of the cultivars Jaraguá and Terezópolis Gigante. Only seeds from the summer cauliflower were used, since it was the self-incompatible line.

Six different periods were tested to sow the maternal line, aiming to reach different levels of flowering time coincidence with the paternal line. Moreover, plants from the paternal line with too early flowering time were removed to avoid their pollinization in the treatments where flowering coincidence was not desired.

The sowing of the winter line (paternal line) was carried out in 02/17/1999, while the sowing for the summer line (maternal line) in 03/05, 03/19, 04/01, 04/05, 29/04 and 05/13/1999. Flower pollination between the lines occurred naturally by pollinator insects, especially bees that were present in the experimental area.

Manual breeding was done before flower anthesis to obtain seeds with guaranteed genetic purity. The hybrid seeds were obtained emasculating the flowers from the maternal line and pollinating them with pollen grains of the paternal line. The maternal line was also self-fertilized for multiplication. After crossing, the branches were protected by plastic bags to avoid contamination.

The experimental design consisted of random blocks with five replicates. Each block had 10 plants with a spacing distance of 2.0 $\times$ 0.4 m. Five most uniform plants were chosen from each block, and the very early or late ones were discarded. The plants from the pollinating line were settled in a proportion of 50% in alternate flower-beds.

Plants were harvested when the silique reached the ideal morphological mature stage, i.e., changing from the green color into the light brown and also turning brittle as the initial process of seed dehiscence. The stalks carrying silique were cut at the plant base and collected individually. Harvesting dates were according to plant maturation in each treatment (T) as follows: 09/15 for T1 and T2; 09/22 for T3 and T4, and 09/27 for T5 and T6. The initial flowering date for each treatment Ws recorded. A treatment was considered to be in flowering period when the blocks showed at least 50% of blooming plants.

Leaf area was measured in one plant per block during the flowering period. Therefore, the plants that represented the average in the block were selected. The destructive method was used, where the plant was collected and leaves measured individually. The total leaf area of the plant was expressed in square centimeters. The apparatus LI-COR model LI-3000 A was used.

By the time of flowering, the number of flowers of three plants per block individually was also registered, obtaining the representative average for each block. After harvesting of five chosen plants per block, the silique of each plant was counted individually, obtaining also the average for each block. The number of seeds produced per plant was also counted, and considering the number of siliques per plant it was possible to estimate the number of seeds per silique.

From each block a lot of 1000 seeds was selected to obtain their dry weight. Seeds were dried in a FANEM oven model 315 SE at 65°C for 72 h when moisture was stable. A standard germination test (SGT) was also applied according to Brasil (1992). Two samples of 50 seeds were collected from each of the 30 original blocks, and stored in gerboxes at a constant temperature of 27°C. The first counting of normal seedlings was done after 5 days, and the second after 10 days. Results obtained from the first SGT counting were used to evaluate seed vigor.

To examine genetic quality, seeds were sown in 30 trays with 128 cells used for each original block in 11/09/1999. From the emerging pool of seedlings, the percentage of contaminant seedlings was determined. Seeds obtained by manual breeding were sown in 2 trays at the same date, confirming that the seeds used in the former were hybrids and in the latter from the maternal line only.

The maternal line seedlings, in this case considered contaminants, presented smaller leaves with remarkable saw-shaped edges and purple colour in their reverse side. Hybrid seedling leaves have larger leaves with less saw-shaped edges and are green in the reserve side. Since vigor difference was found, both seedlings and leaves of the contaminants were notoriously smaller than those of the hybrids.

After the morphological evaluation of the seedlings, they were taken to cold chambers at a constant temperature of 10°C for 10 days. After this the seedlings were transferred to a nursery to count the number of early flowerings. This procedure revealed the contaminant plants because they were originated from maternal line seeds (tropical) and had less requirements in a cold environment. Consequently, they had early flowering.

The data were studied by analysis of variance, and the mean of the treatments were compared by the Tukey test at 5% probability. Results expressed in percentage were transformed into arc sen $\sqrt{x/100}$. The means shown in the result tables are the original ones.

RESULTS AND DISCUSSION

Flowering

Treatments T1 and T2 showed 50% of blooming plants in, respectively, the 15th and 25th of June, 1999 (TABLE 1). These treatments had no coincident flowering time with the paternal line, since the latter was starting to blooming 07 July 99, when the former had already finished flowering. Flowering coincidence with some paternal line plants could be observed only at the end of flowering period, in plants of treatment T3. Since not many paternal line plants were blooming, the minimum of 50% of plants with flowering coincidence between the lines was not found.

Treatment T4 with plants that started blooming on 07 July 99, was the first to show with at least 50% of plants with flowering coincidence between the lines. However, because of the small size of the plants of the treatment 4, when compared to the plants of the treatments T1, T2 and T3, the flowering period also was shorter in this treatment. Consequently, flowering coincidence did not occur with all paternal line plants.

The greater degree of flowering coincidence was found in treatments T5 and T6, blooming on 13 and 15 July 99, respectively. Plants in these treatments had more paternal line pollens available in the environment because, when they started blooming, the paternal line plants were in full bloom.

Despite of the sowing date of T4 having occurred 2 months after the paternal line, they both had the same flowering date (TABLE 1). Plants in T1, T2 and T3 which were sown respectively 15, 30 and 45 days after the pollinator line bloomed before it. These results were already expected since plants in the paternal line are late winter lines while the plants in the maternal line are early tropical lines, under Brazilian growth conditions. Late cultivars need to be exposed to low temperatures for longer periods than early ones flowering induction.

Comparison among treatments showed that the later the sowing date the shorter the treatment cycle. Such observation may be related to the sowing period, when temperatures were decreasing (FIGURE 1). Therefore, the later the sowing the faster the contact of the plants with lower temperature to induce flowering. This procedure reduces the span between sowing and flowering.

Genetic purity – Seedling morpholoical evaluation

Flowering coincidence between paternal and maternal lines showed a great influence in the genetic quality of seeds. The contaminant percentage in the seedling morphological evaluation was very different between treatments (TABLE 2). Treatments T1 and T2 showed the greatest values: 100% and 98.93%, respectively.

These results reflect the complete lack of flowering coincidence between the plants from these treatments and the paternal line plants. Therefore, the pollinator insects worked only in favor of the self-incompatible line, and the plants produced seeds only by breeding among sister plants, i.e., self-fertilization. Therefore, seeds which give rise only to seedlings with the maternal line characteristics, i.e., contaminants were obtained.

In treatment T3 (TABLE 2), there was no complete flowering coincidence with the paternal line plants. However, a small amount of paternal line plants were blooming at the end of the flowering period in T3 and eventually hybrid seeds were produced. In this case, the percentage of contaminant seeds was high (54, 79%), since the majority of flowers in T3 were wide open before a large amount of pollen from the paternal line was available.

In treatments T4, T5 and T6, (TABLE 2), flowering coincidence with the paternal line plants increased. Nevertheless, a gradual increase in paternal pollen availability in the area was observed, causing a decrease in the percentage of contaminant seedlings because the pollinator insects could work between lines.

Plants in T4 were considered to be blooming simultaneously with the paternal lines, because both lines showed only one day of difference when each had 50% of plants blooming. Despite that, a small amount of contaminant seeds was still able to be formed because some maternal line plants bloomed some time before a great amount of paternal pollen was available in the environment.

The treatment T6 was the only one which showed less mean amount of contaminant seedlings, being statistically different from the others, except from treatment T5. Both T6 and T5 presented greatest degree of flowering coincidence, because the paternal line flowering time lasted along, for the whole period of the treatments.

Arús et al. (1982) studied amounts for contaminant seeds in hydrids of different *Brassica oleracea* varying between 1.5 to 40.1% in commercial yields. Comparing these observations with the present data, it may be inferred that such contamination might be even greater when no flowering coincidence is found between the lines. An example for this case was observed for treatments T1 and T2, where the coincidence to occur between the lines was intentionally prevented. Another case was in T3, where the coincidence was very small.

For some genotypes the end of the flowering period should increase even more the degree of plant self-incompatibility. Therefore, the formation of contaminant seeds should be easier (Johnson, 1971).

The results show the importance of the flowering coincidence between plants of paternal and maternal lines for genetic quality of hybrid seeds. It was demonstrated that when there is no flowering coincidence between the lines, or when in a small degree, the amount of contaminant seeds increases significantly. Therefore, the absence of flowering coincidence indicates how inappropriate it is to use the self-incompatibility system to produce hybrid seeds.

To provide commercial cauliflower hybrids, we suggest to plant the maternal line plants in different dates to find what one presents adequate coincidence with the flowering time of the paternal line plants. Moreover, the maternal line plants which do not show flowering coincidence with the paternal lines have to be removed. In the case of reciprocal hybrid production, plant removal has to be done for both parental lines.

Genetic purity – Early flowering test for the seedling

The early flowering test for the seedlings demonstrated the same tendency observed in their morphological evaluation (TABLE 2). Both evaluations showed a decreasing percentage of contaminant seedlings as long as the level of flowering coincidence was increased between the lines.

Statistically, a single difference found was for the early flowering test between the treatments T4 and T5, yet not found in the morphological evaluation.

Leaf area

The earlier the sowing date the larger the leaf area of the plants during flowering (TABLE 3). Plants from

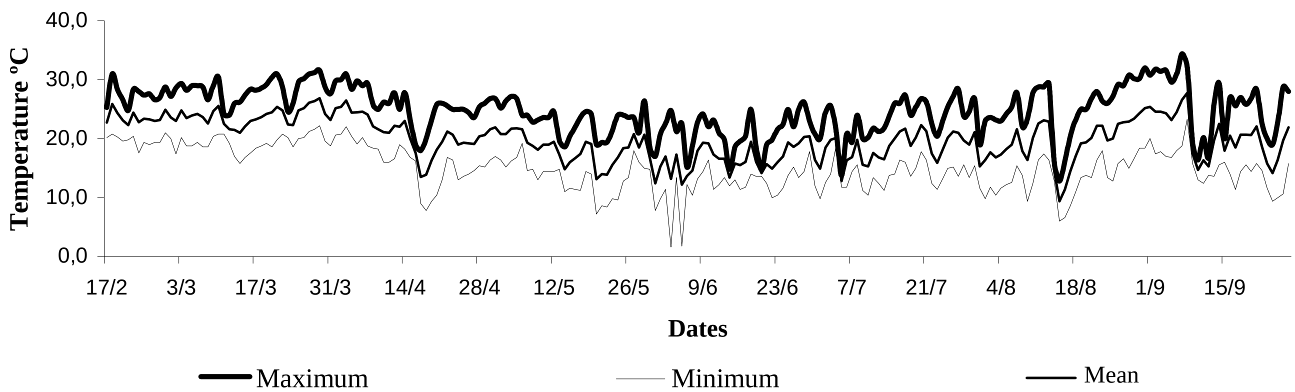

Figura 1 - Maximum, medium and minimum temperatures registered at the experimental area during the period 17/02 to 30/09/1999, Fazenda Lageado, FCA/UNESP, Botucatu, 1999.

treatment T1, the first to be sown, had an average leaf area of 5,634 cm^2. Plants in T2, sowed only 15 days after T1, showed an average leaf area of 2,710 cm^2, i.e., about half of T1.

Examination of the other treatments revealed that the average leaf area continues to decrease gradually accordingly to the sowing date, and T6 was the treatment which showed the smallest leaf area, 482 cm^2. There was statistical difference among almost all treatments, except for T3 which did not present difference in relation to T2 and T4.

According to Booij (1987), the higher the temperature during the initiation stage of cauliflower the greater the number of leaves in the plant. Once the initiation of the head in the growing meristem occurs, the formation of new leaves stops. Since temperature was decreasing at this period (Figure 1), plants from treatments in which seeds were sown earlier longer exposed were to high temperatures. Therefore, these plants had a greater development in the vegetative stage, consequently, forming a larger average leaf area. On the other hand, plants from treatments of late sowing had shorter time to develop themselves before the initiation of the head by the effect of accumulation of low temperatures, forming a reduced leaf area.

Due to the gradual reduction of plants, they could be planted closer to each other, allowing an increased in productivity.

Flower, silique and seed number per plant, and seeds per silique

Similarly to leaf area, the earlier the sowing date the larger the average number of both flowers and siliques per plant (TABLE 3). In treatment T1, the average numbers of flowers and siliques were 3,975 and 898, respectively. In treatment T6, the smallest average number was found, 296 for flowers and 96 for siliques.

Plants from late sowing treatments suffered a rapid flowering induction and stopping leaf formation, consequently, showing small leaf area. A direct consequence of this was the emergence of a small head, and low production of flowers and siliques per plant. Therefore, this indicates that the vegetative stage of development in the plant has a direct influence in productivity. This is in agreement with a previous report (Wurr & Fellows, 1984) in which the authors mention that any reduction of leaf number or size may reduce the mature head size.

For the number of seeds per plant, it was found that the only statistical difference was the smaller plant production of treatment T6 when compared to T4 and T5 (TABLE 3).

A greatest difference was observed between leaf area and flower and silique numbers per plant. It was expected that plants in the treatments with larger silique number would present larger seed number. However, seed number per plant seems not to depend only on the silique number per plant but also on the seed number per silique. Treatments T1, T2 and T3, despite their great silique number per plant, were the ones showing the smallest seed number per silique, i.e., 1.0, 1.0 and 1.2, respectively. Contrarily, plants in treatments T4, T5 and T6 showed an inverse relation, since a smaller silique number per plant was related to a larger seed number per silique. Therefore, the amounts of seed number per plant in the different treatments were almost the same, except for the treatment T6 due to the low silique number found per plant. In this treatment, plants formed a very small leaf area because the of the late sowing, and a very small number of flowers and silique per plant. Although T6 had the larger seed number per silique, it was the treatment with smallest seed number per plant.

Considering the seed number per silique, a gradual and highly significative increase from T1 to T6 was observed. The greater the flowering coincidence between the maternal and paternal line plants the larger the seed number per silique. One of the self-incompatibility characteristics is exactly the reduction in seed number per silique (Bateman, 1954; Watts, 1963; Thompson & Taylor, 1971).

Results obtained in the treatments T1 and T2 demonstrated clearly the above mentioned characteristics. Flowering coincidence among plants in these treatments with the paternal line ones was null. Therefore, the pollinator insects worked only within the maternal line. Because the maternal line plants are self-incompatible, the seed number per silique was reduced significantly.

In the treatment T3, a small increase in seed number per silique due to the small percentage of flowering coincidence with some paternal line plants, small pollen availability was observed in the experimental area. In spite of that the amount of pollen was not enough to differ statistically treatment T3 from T1 and T2.

Treatment T4 presented intermediate seed number per silique, having been statistically different from the others. Since it was a treatment showing flowering coincidence with the paternal line, seed number per silique increased remarkably when compared with the other treatments (more than twice).

The treatments T5 and T6 had the largest amounts of compatible pollen available because the paternal line plants were already in full bloom when the plants in these treatments started to flower. Therefore, seed number per silique was larger in these treatments.

Seed physiological quality

Flowering coincidence between cauliflower lines did not affect physiological quality of the hybrid seeds. No significative differences were found in the dry weight for 1000 seeds for all treatments. The average values in the treatments varied between 4.28 and 4.70 g.

The harvest was performed seed physiological maturation in all treatments, even though it was based

TABLE 1 - Sowing and flowering dates, and number of days from sowing to flowering for the paternal line and the six treatments.

	Sowing date	Flowering date	Day from sowing to flowering
Paternal line	17/02/99	10/07/99	143
T1	05/03/99	15/06/99	103
T2	19/03/99	25/06/99	99
T3	01/04/99	01/07/99	91
T4	15/04/99	09/07/99	84
T5	29/04/99	13/07/99	73
T6	13/05/99	15/07/99	60

T: Differents sowing dates of couliflowers seedlings.

TABLE 2 - Percentage mean values for contaminant seedling observed in morphological evaluations and early seedling flowering of seeds lots obtained in six sowing periods.

Treatment Sowing date	Seedling morphological evaluation	Seedling early flowering
T1:05/03/99	100,00 a	100,00 a
T2:19/03/99	98,93 a	100,00 a
T3:01/04/99	54,79 b	61,98 b
T4:15/04/99	14,72 c	21,37 c
T5:29/04/99	6,10 cd	11,35 d
T6:13/05/99	4,75 d	5,33 d
CV%	11,06	7,12

T: Differents sowing dates of couliflowers seedlings Mean values followed by the same letter in the column do not differ statistically according to Tukey´s Test at 5%.

TABLE 3 - Mean leaf area, number of flowers, silique and seeds per plant, and number of seeds per silique observed for all treatments.

Treatment Sowing date		Flower umber per plant	Silique number per plant	Seed number per plant	Seed number per silique
	cm2				
T1:05/03/99	5.634 a	3.975 a	898 a	1.095 ab	1,00 c
T2:19/03/99	2.710 b	2.722 b	672 ab	1.080 ab	1,00 c
T3:01/04/99	2.181 bc	2.504 c	617 ab	1.221 ab	1,20 c
T4:15/04/99	1.884 c	1.777 c	412 bc	1.347 a	2,80 b
T5:29/04/99	1.266 d	696 cd	207 c	1.447 a	6,40 a
T6:13/05/99	482 e	296 d	96 c	701 b	7,00 a
CV%	2,84	14,64	32,94	26,29	24,54

T: Differents sowing dates of couliflowers seedlings means followed by the same letter in the column do not differ statistically according to Tukey´s Test at 5%.

on morphological features. It is also clear that the weight of 1000 seeds does not depend directly on seed number per silique, neither does plant leaf area. The latter were found to differ greatly among the treatments.

Seeds from all treatments had mean indexes for germination superior to 90% (maximum 95.05%), except in T4 which was 86.97%. However, no significative differences were found among treatments.

In the first measurement for the germination standard test, which was used as a vigor test, no statistical differences were observed among the treatments. The mean values of the treatments varied between 87 and 93%.

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