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MEIOTIC STUDIES AND POLLEN STAINABILITIES OF F1 HYBRIDS BETWEEN Capsicum baccatum x C. eximium AND C. baccatum x C. cardenasii

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Abstract

Meiotic studies have been conducted on F1 hybrids between *Capsicum baccatum* x *C. cardenasii* and *C. baccatum* x *C. eximium*. Meiotic studies with conventional squashes in *Capsicum* are sometime troublesome, since chromosomes can be sticky so that it is dfficult to get a good separation of chromosomes. It was therefore decided to study synaptonemal complexes at pachytene, as well as conventional squashes at diakinesis or metaphase. In this article, problems observed during these kind of studies were noted and possible explanations were given.

Capsicum baccatum x C. eximium ve C. baccatum x C. cardenasii F1 Hibridleri Üzerinde Polen Boyanabilirliliği ve Mayoz Çalışmaları

Capsicum baccatum x *C. eximium* ve *C. baccatum* x *C. cardenasii* arasında yapılan melezlemeler sonucu elde edilen F1 hibridler üzerinde mayoz ve polen boyaması çalışmaları yapılmıştır. Kromozomların birbirine yapışık durmasından ve bu yüzden iyi ayrıştırılamamasından dolayı klasik ezme yöntemleriyle yapılan mayoz çalışmaları *Capsicum*'da iyi sonuç vermez. Bu yüzden diakinez veya metafaz aşamalarında yapılan klasik mayoz çalışmalarının yanısıra pakiten aşamasında synaptonemal kompleks çalışmalarının yapılmasına karar verilmiştir. Bu makalede yapılan çalışmalar süresince gözlenen problemler belirtilmiş ve bu problemlerin nedenleri açıklanmaya çalışılmıştır.

Introduction

Interspecific F_1 hybrid sterility has been known in *Capsicum* for a long time and it has been demonstrated that interchanges (reciprocal translocations) are responsible for at least part of the sterility of the hybrids both within and between the species (1,2). Two breaks may occur in non-homologous chromosomes which may rejoin to give translocated chromosomes.

Koompai (3) studied meiosis in the F_1 hybrid between an accession of domesticated *C. annuum* and a weedy accession of the same species and found that they differ by one reciprocal interchange. In the same study, he also found

that one accession of the white-flowered *C. frutescens* was distinguished from an accession of the purple-flowered *C. pubescens* by two interchanges.

Tanksley (4) showed that F_1 hybrids between *C. chinense* and *C. annuum* were heterozygous for one interchange.

Haji Itam (5) working on one of the F_1 hybrids used in this study, *C. baccatum* accession Hawkes 6489 x *C. cardenasii* accession SA268, showed that it was heterozygous for one interchange. Pickersgill (personal communication) found that there were no multivalents in the F_1 hybrid between *C. cardenasii* SA268 and *C. eximium* Hawkes 3860 which means that both species have the same chromosomal end arrangement. Gonzalez de Leon (6) reported that the interspecific F_1 between *C. baccatum* Hawkes 6489 and *C. baccatum* SA219 had 12 bivalents which means both accessions have the same chromosomal end arrangements. So the F_1 hybrid between *C. baccatum* x *C. eximium* is likely to be heterozygous for one interchange.

In an individual heterozygous for one interchange, two pairs of chromosomes are usually associated in a ring or a chain at meiosis (7). The pairing of homologous portions of this group of 4 chromosomes results in cross shaped configuration at pachytene and this cross shape opens up into a complex of four chromosomes associated mainly at the ends at diakinesis and metaphase 1.

The type of orientation and the number of chiasmata formed will affect the conformation of the quadrivalent at metaphase 1 and subsequent separation of chromosomes involved in the interchange. If alternate chromosomes in the quadrivalent are directed toward the same pole (alternate orientation) separation at anaphase 1 usually produces viable gametes. If adjacent chromosomes in the quadrivalent are directed towards the same pole (adjacent orientation) separation at anaphase 1 will produce gametes which contain duplications and deficiencies. Many of these gametes will be inviable. If alternate and adjacent orientation occur with equal frequency in the pollen mother cells, plants heterozygous for one interchange will produce up to 50% viable and 50% inviable pollen. Therefore, individuals heterozygous for an interchange can be recognised by a reduction in percent viable pollen (8).

When an F_1 heterozygous for one interchange and for various other genetic loci is backcrossed to one parent, the resulting backcross progeny will consist of some plants which are heterozygous for the interchange and partially sterile and other plants which are homozygotes for the interchange and fully fertile. These backcross plants will also include some plants which are homozygous and some which are heterozygous for other genetic loci. "If a segregating gene is near the breakpoint of the interchanged chromosomes, then plants heterozygous for the gene will be heterozygous also for the interchange, hence partially sterile. Plants which are homozygotes for the gene will also be interchange homozygotes, hence fully fertile. The two phenotypic classes for the segregating gene will therefore differ in mean pollen stainability" (9). However, if the gene is not

located near the breakpoint of the interchanged chromosomes, then mean pollen stainability between the two classes will not differ significantly (8).

Meiotic studies with conventional squashes in *Capsicum* are sometimes troublesome, since chromosomes can be sticky so that it is difficult to get a good separation of chromosomes. It was therefore decided to study synaptonemal complexes at pachytene, as well as conventional squashes at diakinesis or metaphase 1.

Additionally, synaptonemal complexes studies could have been helpful to see how many chiasmata occur, since observing the number of chiasma very accurately in conventional squashes is rather difficult. Apart from that, they could have determined the position of chiasmata whether proximal or all along the arms.

Materials and Methods

Techniques For the Study of Meiotic Chromosomes

Squash Preparations

Young flower buds of the F_1 hybrid between *C. baccatum* (SA219) and *C. eximium* (Hawkes 3860) were fixed in a modified Carnoy's fluid (6:3:1, Alcohol: Chloroform: Acetic acid) and stained in Snow's carmine (10). Pollen mother cells were squeezed out of anthers and meiotic observations were made on cells.

Two Dimensional Spreads of Synaptonemal Complexes

Stack's (11) technique was applied for two dimensional spreads of synaptonemal complexes. Anthers from buds determined by the squash technique to contain primary microsporocytes at stages of prophase from leptotene through pachytene were placed in a cavity slide. The depression contained 0.1 ml. of a solution that was 0.8 M sorbitol, 1% Polyvinyl-pyrrolidone (PVP), 0.6 mM KH_2PO_4, 1.0 mM $CaCl_2$, 0.1 mM PIPES, 1.6 mM $MgCl_2$ and 0.3% potassium dextran sulfate. The pH was adjusted to 5.0 with 0.1 M KOH.

The anthers were cut with a small scalpel blade and contents of the anthers were gently squeezed out with a dissecting needle. The anther walls were removed and approximately 1 mg. of β 3-glucuronidase was added. This digestion medium was briefly stirred to dissolve the enzyme and then the slide was transferred to a covered
petri dish containing moist filter paper and kept at room temperature. Samples were
kept in digestion solution for 15 min., 30 min., 1 hr., 1 hr. 30 min., 2 hr., or 2 hr. 30 min. to find out optimal digestion duration for *Capsicum*.

Following incubation, approximately 5-10 µl of the cell suspension was placed on a plastic coated slide (a 1% plastic solution was made by dissolving 1 g of plastic in a 100 ml of chloroform) by pipette. The cell suspension was covered with a coverglass and an absorbent tissue i.e. Whatman filter paper was placed against the one edge of the coverglass. After that, 2.5-5.0 ml. of distilled water was

placed on the other side of the coverglass and water was pulled under the coverglass. The coverglass was removed by the dry ice method (11). The slides were air dried briefly then fixed for 15 min., 30 min., 1 hr., 1 hr. 30 min., 2 hr., or 2 hr. 30 min. in a 4% solution of formaldehyde buffer with borate buffer at pH 7.0 to find out the optimal fixation time for *Capsicum*.

After fixation, the preparations were stained in 50% (w/v) silver nitrate solution and staining was monitored under a light microscope.

Pollen Stainability

Pollen of two F_1 hybrids, their parental accessions and backcross plants of *C. baccatum* x F_1 (*C. baccatum* x *C. eximium*) and *C. baccatum* x F_1 (*C. baccatum* x *C. cardenasii*) were stained to get a rough estimate of pollen viability. It was not possible to study pollen stainability of plants from the backcross F_1 (*C. baccatum* x *C.eximium*) x *C. eximium*, since none of the backcross plants had anthers which dehisced.

To test pollen stainability, pollen was collected from flowers whose anthers had dehisced on the day of collection. One flower per plant and three plants per accession and each backcross plant were examined. Pollen grains were stained with 0.1% (w/v) cotton blue in lactophenol (29g phenol, 1g cotton blue (C.I. 42755), 25ml water, 25ml lactic acid and 25ml glycerol) for 3-24 hours and observed under a light microscope. Stained and unstained pollen grains were counted in a sample of at least 200 pollen grains per flower. The Least Significant Difference (LSD) test was applied for means separation at 0.05% significance level. The statistical analyses were carried out by using the SAS computer package (SAS Institute Inc. 1985, Cary, North Carolina, USA).

Pollen stainability was tested three times for *C. baccatum* accession SA219 and *C. eximium* accession Hawkes 3860 and their F_1 hybrid in 1992, while *C. baccatum* accession Hawkes 6489, *C. cardenasii* SA268 and their F_1 hybrid were tested in 1994 as follows:

- Early summer (15th. May)
- Middle summer (15th. August)
- Autumn (15th. October)

Pollen stainability of both backcross combinations *C. baccatum* x F_1 (*C. baccatum* x *C. eximium*) and *C. baccatum* x F_1 (*C. baccatum* x *C. cardenasii*) was tested between June and August in 1994.

Results

Meiotic Studies

Observations on preparations of pollen mother cells squashed in Snow's carmine showed that chromosomes were sticky, making counting or observing the pairing of chromosomes very difficult. At the beginning, it was thought that heat or water stress might be responsible. Plants were therefore kept in different

temperatures (15 and 25°C) in controlled temperature rooms. Bowls with a constant supply of water were kept underneath the pots to overcome any water stress. Although presence of univalents was noted on some preparations of pollen mother cells, most of the time chromosomes remained sticky and clumped.

Attempts to observe synaptonemal complexes were not successful either. Removing the walls of the pollen mother cells was very difficult. Although the walls were occasionally removed by keeping the slides in the digestion solution for a long time (2hr. 30 min.), at the end of this time it was not possible to see any synaptonemal complexes. Keeping the slides in the digestion solution for a long time probably had an adverse effect on the complexes. Because time was limited, this investigation had to be abandoned.

Pollen Stainability

Results from pollen stainability studies for parental accessions and their respective F_1 hybrids are presented in Tables 1 and .2.

Mean pollen stainabilities of *C. baccatum* accession Hawkes 6489 in May, August and October differed significantly ($p= 0.05$). However mean pollen stainabilities of *C. cardenasii* and the F_1 hybrid (*C. baccatum* x *C. cardenasii*) were not significantly different in May, August and October.

Mean pollen stainabilities of *C. baccatum* accession SA219 and *C. eximium* in August and October did not differ significantly, but in both species mean pollen stainability in May was significantly higher than in August and October. But mean pollen stainability of the F_1 hybrid (*C. baccatum* x *C. eximium*) differed significantly in May, August and October.

Pollen stainability of backcross plants *C. baccatum* x F_1 (*C. baccatum* x *C. eximium*) and *C. baccatum* x F_1 (*C. baccatum* x *C. cardenasii*) showed a continuous range from as high as that of the parent to as low as 30%. 6 of the backcross plants had mean pollen stainabilities below 50% and these backcross plants are likely to be heterozygous for the interchange.No plant in the progeny of either backcross had a mean pollen stainability as low as that of the F_1

Discussion

It was not possible to confirm the expected interchange heterozygosity of the F_1 hybrid *C. baccatum* (SA219) x *C. eximium* (Hawkes 3860). This hybrid is quite likely to be heterozygous for one interchange like the hybrid *C. baccatum* (Hawkes 6489) x *C. cardenasii* (SA268) studied by Haji Itam (5).

Although synaptonemal complex studies were not successful in this study, it does not necessarily mean that this method does not work at all for F_1 hybrids in *Capsicum*. The main problem in this present study was time limitation. In future, other researchers with plenty of time may try several points to improve this technique for *Capsicum*. For example it may be necessary to find out optimal digestion and fixation time. In the literature, for example Stack (11) used 15 min.

Table 1. Mean Pollen Stainability (%) of *C. baccatum*, *C. cardenasii* and F_1 Hybrid (*C. baccatum* x *C. cardenasii*) In Different Months

Month (1994)	*C. baccatum* Hawkes 6489	*C. cardenasii* SA268	F_1 (*C. baccatum* x *C. cardenasii*
May	90.74 ***b*** (*)	87.79 ***a***	13.57 ***a***
August	93.25 ***a***	86.14 ***a***	12.76 ***a***
October	89.15 ***c***	85.29 ***a***	13.07 ***a***
Mean	91.05	86.38	12.88

(*) Means with same letter within a column are not significantly different at p= 0.05

Table 2. Mean Pollen Stainability (%) of *C. baccatum*, *C. eximium* and F_1 Hybrid (*C. baccatum* x *C. eximium*) In Different Months

Month (1992)	*C. baccatum* SA219	*C. eximium* Hawkes 3860	F_1 (*C. baccatum* x *C. eximium*
May	84.40 ***a*** (*)	78.49 ***a***	18.98 ***a***
August	71.43 ***b***	76.66 ***b***	14.42 ***b***
October	71.23 ***b***	76.42 ***b***	11.48 ***c***
Mean	75.61	77.94	14.78

(*) Means with same letter within a column are not significantly different at p= 0.05

and 30 min. digestion and fixation times for *Lycopersicon*. When these duration times were applied to *Capsicum*, the pollen walls were still present at the end of the digestion time. This result may indicate either that this digestion time was not long enough for *Capsicum* or that the enzyme used in *Lycopersicon* was not the optimal one to remove pollen walls of *Capsicum*. Thus, it may be necessary to try different enzymes.

Since one of the main problems was to remove pollen wall, it was thought that it may be helpful to apply some of the tissue culture protocols used for culture of pollen protoplasts in which removing the pollen wall is essential. In this present study, one part of the protocol to remove the pollen wall has been tried, but it was not successful either. In future work, trying some of these protocols may be helpful.

Although neither of the techniques worked for one F_1 hybrid combination, it might have been possible to get information about interchanges by studying meiosis in some of the backcross plants. In the backcross combination *C. baccatum* x F_1 (*C. baccatum* x *C. eximium*), 6 of the backcross plants had mean pollen stainabilities below 50% and these backcross plants are quite likely to be heterozygous for the interchange. Thus, meiotic studies on these few backcross plants could have given some information about interchange.

As stated earlier heterozygosity for an interchange can partly explain the values for pollen stainability obtained in the two F_1 hybrids. In addition, poor chromosome pairing and consequent presence of univalents may reduce pollen stainability. When conventional squash method was used to study pollen mother cells at diakinesis or metaphase 1, in some of cells univalents were observed. This point of view can also be supported by checking pollen stainabilities of backcross plants. Since none of the plants had mean pollen stainability close to the F_1 hybrid, it can be assumed that backcrosses to *C. baccatum* caused better chromosome pairing and reduced number of the univalents.

Most of the backcross plants have pollen stainability above 50% but none had pollen stainability close to the F_1 parents. When F_1 plants which are heterozygous for an interchange are crossed to *C. baccatum*, half of the backcross plants are expected to be heterozygous for the interchange and produce up to 50% inviable gametes, while the other half of the progeny are expected to be homozygous for the interchange and produce more than 50% viable gametes. The results obtained suggest that the interchange in the backcross plants is showing a distorted segregation ratio, skewed in favour of the *C. baccatum* end arrangement.

As can be seen from Table 1 and .2, some differences were observed among parental accessions and their respective F_1 hybrids in different months. However, conclusions are difficult to draw, since these pollen stainability studies have been made in two different years.

Furthermore, although these differences were significant statistically, they do not necessarily mean any biological significance.

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