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Pollen Grain and Hybridization Studies in the Genus *Capsicum*

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Abstract

The current study aimed to evaluate the pollen viability of the commonly cultivated varieties of *Capsicum* species and assessed the potentials for gene exchange among the genotypes through hybridization studies. *Capsicum annuum* var. *abbreviatum*, *C. annuum* var. *acuminatum*, *C. annuum* var. *grossum* and *C. frutescens* var. *baccatum* were the species and varieties used in this study. The present findings indicated that the percentage of pollen viability varied in the studied *Capsicum* genotypes. The highest pollen viability was obtained in *C. annuum* var. *abbreviatum* (96.3%), followed by *C. annuum* var. *grossum* (95%), and *C. annuum* var. *acuminatum* (91.1%). The lowest pollen viability was recorded in *C. frutescens* var. *baccatum* (86.2%). The pollen viability was high in most varieties indicating that meiosis is normal, resulting in viable pollen grains. Several intraspecific and interspecific crosses were performed among the *Capsicum* genotypes and three putative hybrid fruits were produced. Percentage successes obtained in the crosses were low and comparable in both intra and inter-specific crosses. In the entire crosses pattern, pollination success of 10% was recorded for *C. frutescens* var. *baccatum* and *C. annuum* var. *acuminatum*. Knowing the nature and viability of pollen grains may help in predicting the success rate of hybridization and the successful crosses between *C. frutescens* var. *baccatum and C. annuum* var. *acuminatum* suggest that these two varieties are the closest genetically.

Keywords: emasculation, gene exchange, interspecific crosses, intraspecific crosses, pollen viability

Introduction

The *Solanaceae* family comprises many economically important food and industrial crops such as potato, tobacco, tomato, garden egg, petunia and pepper (*Capsicum*). *Solanaceae* is an important source of almost 300 alkaloids (Al-Wadi, 2007). Solanine, scopolamine, atropine and hyoscyamine are the key alkaloids of the family (Kalifa *et al*., 1998).

The genus *Capsicum* consists of approximately 22 wild and 5 domesticated species (Bosland and Votava, 2000). The domesticated species include *C. annuum*, *C. frutescens*, *C. chinenses*, *C. baccatum*, and *C. pubescens* (Bosland and Votava, 2000). Most *Capsicum* fruits are pungent, because the placenta accumulates capsaicinoids (e.g., capsaicin) an alkaloid that is a digestive stimulant, and an important ingredient of daily diet with many other medicinal properties (Zewdie and Bosland, 2001; Thompson *et al*., 2005). *Capsicum* species can be divided into several groups based on fruit/pod characteristics such as pungency, colour, shape, flavour, and size (Bosland and Votava, 2000). Despite their vast trait differences most cultivars of peppers commercially cultivated belongs to the species *C. annuum* L. (Smith *et al*., 1987; Bosland, 1992). The fruit colour is due to the presence of total carotenoid pigments which consists mainly of capsanthin and capsorubin in red fruits and β– carotene and violaxanthin in yellow-orange fruits (Bosland and Votava, 2000; Kumar *et al*., 2003). The pharmaceutical utilization of capsaicinoids is attributed to its antioxidant, anticancer, antiarthritic, and analgesic properties (Bosland and Votava, 2000). In addition, pepper fruits are valuable due to their richness in ascorbic acid (Bosland and Votava, 2000).

Pepper is one of the major revenue sources in Nigeria and in the world, it is most crucial and most used condiment (Showemimo and Olanrewaju, 2000). In order to improve the *Capsicum* productivity, one way is to create a superior pepper genotype. The development of a basic population to create the novel varieties can be conducted in several ways, e.g. through hybridization or crossbreeding since it attains the direct results, such as genetic variability of progeny, heterosis and fixation of desirable traits (Zecevic *et al*., 2003). Diallel crossing, as one of the methods enables evaluation of parents and gives information regarding the genetic control of quantitative traits which is important for choosing breeding methods (Zecevic *et al*., 2003). Interspecific hybridization is important in plant breeding as a tool for gene transfer from one genotype, usually a wild species, to another, cultivated genotype that takes the desired gene (Hajjar and Hodgkin, 2007). However, the cross between species may be inconsistent or incompatible due to the existence of a set of pre- and post-zygotic barriers (Hajjar and Hodgkin, 2007). Pre-zygotic barriers can be: the absence of pollen grain germination and the delay or inhibition of pollen tube growth (Hajjar and Hodgkin, 2007). After fertilization, the main barriers are embryonic death due to endosperm

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Table 1. Description of pepper plants used in this study

Variety	Voucher Number	Description
C. annuum var. *abbreviatum* Fingerh	UIH 001/532	Medium sized annual plant, small, round and wrinkled fruit with hot taste, one pedicel per node
C. annuum var. *acuminatum* Fingerh	UIH 002/532	Medium sized annual plant, long pointed and pendant fruit with hot taste, one pedicel per node
C. frutescens var. *baccatum* (L.)	UIH 004/751	Large perennial shrubs; small pointed fruits with very hot taste; 2-4 pedicel per node.
C. annuum var. *grossum* (L.)Sendt	UIH 003/532	Small annual plant, very large bell-shaped fruit with mild taste; one pedicel per node.

Table 2. Pollen viability in the studied *Capsicum* genotype

Varieties	Total no. of pollen	No. of viable pollen	No. of non-viable pollen	% viable pollen	% non-viable pollen
C.annuum var. *abbreviatum*	353	314	13	96.3	3.7
C.annuum var. *acuminatum*	449	409	40	91.1	8.9
C.annuum var. *grossum*	518	491	27	95	5
C.frutescens var. *baccatum*	551	475	76	86.2	13.8

degeneration and the total or partial sterility of hybrid plants. These barriers prevented the use of the wild species carrying important genes that are absent in the cultivated forms in breeding programs (Hajjar and Hodgkin, 2007).

In *Capsicum*, usually no interspecific hybrids involving species belonging to different gene complexes have been obtained due to some incongruity aspects: incompatibility between species, unilateral incompatibility and abortion of the embryo after fertilization and male sterility. Moreover, the more distant two species are genetically, more sterile or unviable their hybrid will be (Onus and Pickersgill, 2004).

One possible post-zygotic barrier can be hybrid male sterility, which can be measured by the viability of pollen grains in the hybrid combination. The pollen viability is an important indicator of the ability of pollen grains to germinate on the flower stigma and fertilize the egg cell, which is a decisive stage in fertilization (Dafni, 1992). Information on pollen viability is essential for studies on the reproductive biology of plant species, allowing the confirmation and understanding of results reported of certain crosses. Pollen viability can be estimated by a number of methods: Lugol solution, Fluorochromatic Reaction (FCR), vital dyes such as tetrazolium salt, germination test in vivo and in vitro and Alexander´s solution. According to Alexander (Alexander, 1969), the staining solution is a differential dye that distinguishes viable and unviable pollen grains in most angiosperms, and is considered a quick, cheap and easy technique (Dafni, 1992).

The present study evaluates pollen viability of the commonly cultivated varieties of *Capsicum* species and assesses the potentials for extent of gene exchange among the varieties.

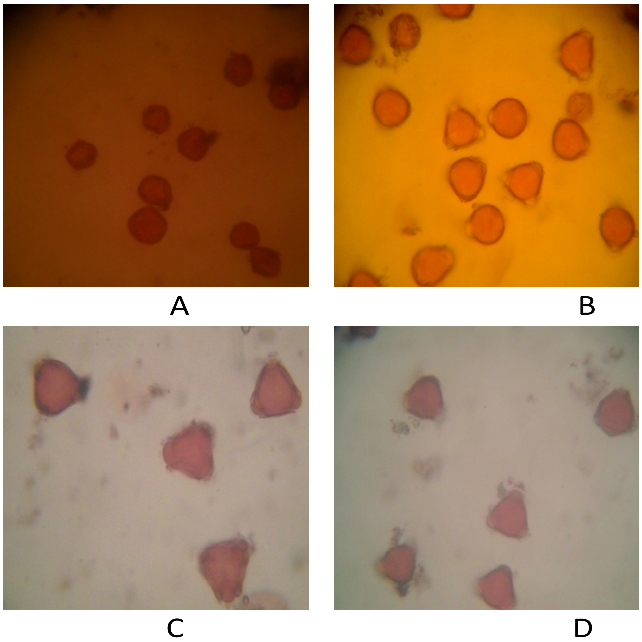

Fig. 1. Variation in pollen viability, size and shape in the studied pepper varieties. A-*C. annuum* var. *abbreviatum*; B-*C. annuum* var. *acuminatum*; C-*C. annuum* var. *grossum*; D-*C. frutescens* var. *baccatum*. MG X 40

Materials and Methods

The study was carried out at University of Ilorin (UNILORIN) Botanical Garden between November 2013 and May 2014. The four pepper varieties (Table 1) used were obtained from Ilorin, Kwara State, Nigeria (latitude 8° 30´N and longitude 4° 33´E).

Pollen grain study

Evaluation of pollen viability followed the method reported by Alexander (1969). Pollen viability was determined by aceto-carmine stainability. Flower buds were collected daily at anthesis at about 10.00 a.m. The anthers obtained from the flower buds were squashed in drops of aceto-carmine stain on a clean glass slide and covered with clean cover slid. The slides prepared were examined at × 40 magnification of Olympus light microscope. The fully/deeply stained pollen grains with spherical shape were considered viable, while the unstained pollens were taken as non-viable/ sterile. The number of viable and non-viable pollen grain was counted and percentage pollen viability was determined.

Interspecific and intraspecific hybridization

In order to study the compatibility of the four pepper genotypes, inter and intraspecific crosses were made. At maturity, the four varieties were subjected to a full diallel and reciprocal cross. Controlled pollination was carried out in emasculated flower buds prior to anther dehiscence. This was performed by opening and emasculating flower buds one day before anthesis.

Table 3. Results of intraspecific and interspecific hybridization of the studied *Capsicum* varieties

Cross combination	Number of flowers	Pollination success (%)
Interspecific diallel crosses		
C. annuum var. *abbreviatum*× *C. frutescens* var. *baccatum*	25	0
C. annuum var. *acuminatum*× *C. frutescens* var. *baccatum*	15	0
C. annuum var. *grossum*× *C. frutescens* var. *baccatum*	20	0
Interspecific reciprocal crosses		
C. frutescens var. *baccatum*× *C. annuum* var. *abbreviatum*	25	0
C. frutescens var. *baccatum*× *C. annuum* var. *acuminatum*	30	10
C. frutescens var. *baccatum*× *C. annuum* var. *grossum*	15	0
Intraspecific diallel crosses		
C. annuum var. *abbreviatum*× *C. annuum* var. *acuminatum*	25	0
C. annuum var. *abbreviatum*× *C. annuum* var. *grossum*	15	0
C. annuum var.*acuminatum*× *C. annuum* var. *grossum*	20	0
Intraspecific reciprocal crosses		
C. annuum var. *acuminatum*× *C. annuum* var. *abbreviatum*	25	0
C. annuum var. *grossum*× *C. annuum* var. *abbreviatum*	15	0
C. annuum var. *grossum*× *C. annuum* var. *acuminatum*	15	0

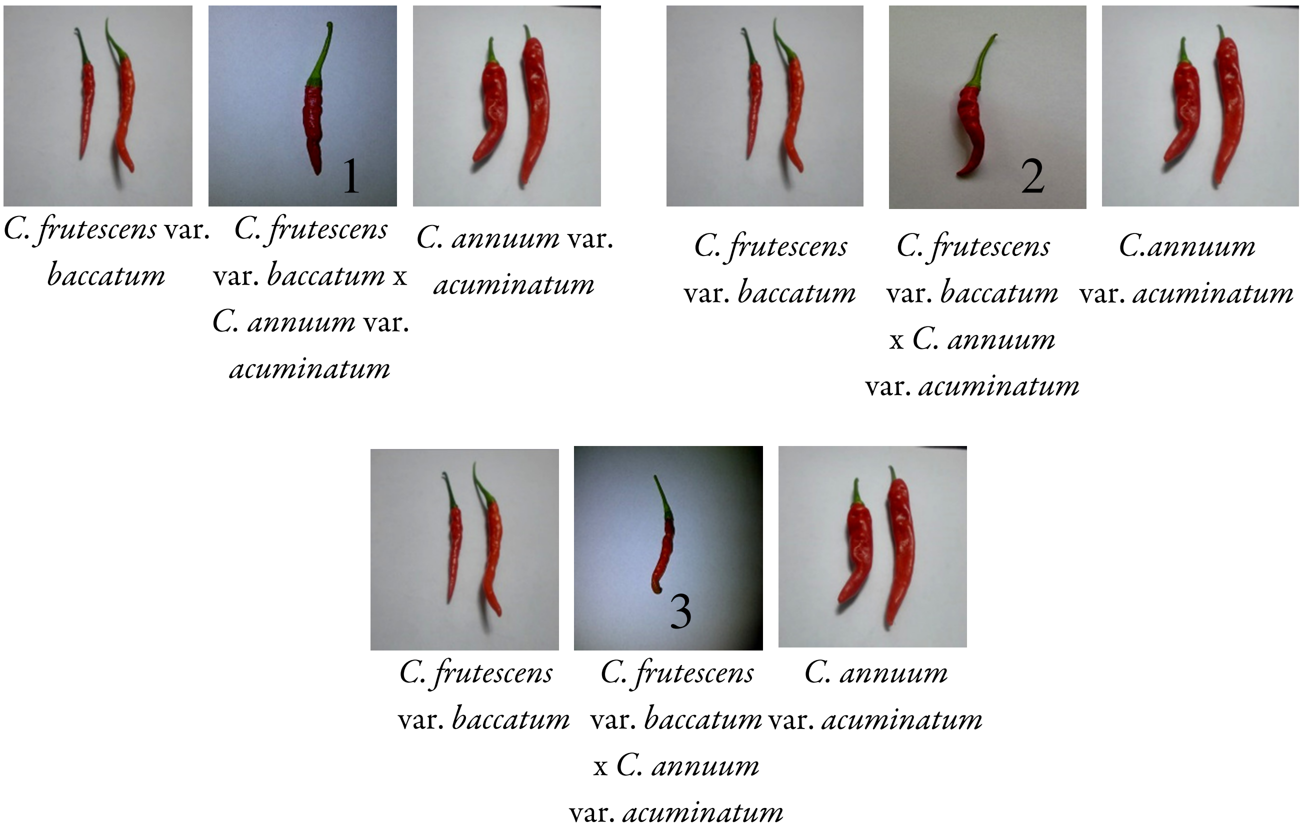

Fig. 2. The fruits of parental genotypes and the hybrids. Parents: *C. frutescens* var. *baccatum* and *C. annuum* var. *acuminatum*

These flower buds were recognized by their characteristic size and tension along the seams of petals. Pollen grains from desirable sources of newly opened flowers were rubbed/dusted directly on the stigma of the emasculated flowers. Thereafter, the pollinated flowers were protected in paper bags to prevent undesirable pollen contamination and the crosses tagged. The data obtained from pollen grain study and hybridization studies were converted into percentage (%).

Results

Pollen viability study

The distinction of viable (fertile) from non-viable (infertile) pollen grains was performed with high accuracy. The results indicated that the percentage of viable pollen varied in the studied *Capsicum* genotypes. The highest pollen viability was obtained in *C. annuum* var. *abbreviatum* (96.3%), followed by *C. annuum* var. *grossum* (95%), and *C. annuum* var. acuminatum (91.1%). The lowest rate of pollen viability was recorded in *C. frutescens* var. *baccatum* (86.2%) (Table 2).

In addition, the pollen viability of the four studied pepper genotypes was high, indicating that these varieties have a normal meiosis and are therefore were well-adapted to the local environment. Pollen shape in the four varieties varied in size and shape. The shape varied from uniporate to triporate (Fig. 1).

Hybridization study

The results of intraspecific and interspecific crosses are summarized in Table 3. The success of artificial hybridization in almost all the patterns were low both in intra and in interspecific crosses. In all the crosses, pollination success of 10% was recorded for *C. frutescens* var. *baccatum* × *C. annuum* var. *acuminatum*, as three hybrid fruits were obtained from the 25 flowers pollinated, while zero success was recorded in all others including crosses among the varieties of *C. annuum* (Fig. 2). This is most likely due to low number of pollinators.

Discussion

The pollen viabilities of the four varieties of pepper were high, indicating that these genotypes had normal meiosis and were therefore fertile, but successful crossing among them depends on their relatedness genetically. For example, intervarietal cross was only successful between varieties of *C. frutescens* var. *baccatum* and *C. annuum* var. *acuminatum,* both having similar pollen shape indicating phylogenetic similarity, whereas, crossing was not successful between plants of dissimilar pollen shapes. This underscores the need for genetic similarities in successful crossing. Carlos *et al.* (2001) reported high pollen viability in some cultivars of *Capsicum annuum*. This finding indicates that the non-formation of fruit or the formation of seedless fruits observed in some interspecific crosses established and described in literature cannot be attributed to pollen viability alone (Carlos *et al.,* 2001). Factors influencing hybrid formation rates include genetic similarities, pollen viability, the presence of suitable pollination vectors, efficiency of seed set and seed viability (De Vries *et al*., 1992).

Recently, considerable attention is given to a number of these factors, but the influence of pollen viability has received relatively little attention (De Vries *et al*., 1992). However, given the importance of pollen development and function in sexual plant reproduction, it is obvious that pollen viability is a prerequisite for hybrid production (Groot *et al*., 2003). Therefore, knowledge of the nature and viability of pollen grains may help in predicting hybrid formation (Groot *et al*., 2003).

Another important consideration in hybridization is the incompatibility status of the parents which may favour specific cross combinations as found in accessions of *Cola nitida* by Morakinyo (1981).

The studied *Capsicum* species showed variation in fruit set. The percentage fruit set was higher when *C. frutescens* var. *baccatum* was hybridized with *C. annuum* var. *acuminatum* than the other *Capsicum* species. The low hybridization success recorded in the study indicates the difficulties of crossing within and between species. These results are in agreement with the report presented by Campos *et al.* (2005), where hybrid combinations of *C. chinense* and *C. annuum* produced either no fruits or fruits with no viable seeds, as observed by Pickersgill (1993) as well. Falusi and Morakinyo (1994) also reported low percentage success (0%-11.4%) in both inter and intra specific cross in the genus *Capsicum*. The percentage fruit set observed in this study were lower than reported by Ribeiro and Melo (2005) for the cross between genotypes of *C. chinense* with *C. annuum* with fruit set rate of 73.5% to 100%, using *C. chinense* as female parent and 87.5% to 91.5% in reciprocal crosses. The data, however, do not provide enough evidence to conceive that the collections are not biologically related or that they are reproductively isolated.

On the other hand, the 10% successful crosses between *C. frutescens* var. *baccatum* × *C. annuum* var. *acuminatum* showed that hybridization is possible in nature and is most probably responsible for the existence of many varieties of pepper that are in cultivation. This corroborates the work of Falusi and Morakinyo (1994). There were little differences in the percentage pollen viability among the pepper genotypes studied but there were great differences in fruit set in reciprocal crosses indicating incipient dioecy as described by Morakinyo (2011).

Conclusion

The studies revealed that inter and intraspecific cross in *Capsicum* is possible and can lead to obtain viable seeds and fruits. The successful crosses between *C. frutescens* var. *baccatum* × *C. annuum* var. *acuminatum* suggest that these two varieties are the closest genetically.

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