Assessment of natural cross-pollination levels in chili pepper (Capsicum annuum L.)

A W Ritonga¹, M Syukur¹, Rahmi Yuniandi¹, and Sobir¹

¹ Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Bogor, West Java, Indonesia.

Email: muhsyukur@yahoo.com

Abstract. Inconsistency of the current information on the natural cross-pollination (NCP) rates of chili pepper stimulated this research to update the natural cross-pollination rates of chili pepper. The objective of this study was to determine the level of natural cross-pollination and the various factors that influence the NCP in chili pepper. The experiment used four genotypes of chili peppers, “IPBC2, IPBC5, IPBC120” (green hypocotyl) and IPBC20 (purple hypocotyl). The experiment utilized the dominance of purple hypocotyl colors over green hypocotyl colors to determine NCP. The experimental field consisted of three blocks of trial. Each block was planted by purple hypocotyl genotype in the central plot and green hypocotyl genotypes in the surrounding four plots. Seeds were harvested from each plant and were examined for the levels of NCP in the next generation. The results showed that there were differences on percentages of NCP on different chili pepper genotypes. The results also showed that the levels of NCP in chili pepper could be associated with stigma exertion length and the level of successful artificial cross-pollination.

Keywords: Artificial cross-pollination, heterosis, inbreeding depression, purple hypocotyl, and stigma exertion.

1. Introduction

Information on natural cross-pollination (NCP) rates of a crop species in specific environment is important when choosing a breeding method for crop improvement, germplasm maintenance and commercial seed production. Chili pepper is known as self-pollinated crops [1]. Self-pollinated crops are plants which have NCP rates under 10%. Chili pepper has a perfect flower. The time of anthers dehisce is almost the same time with the receptive stigma. Pollination occurs after the flower open in chili pepper.

There were variations in NCP in chili pepper. [2], [3], and [3] reported that the NCP rates in chili pepper could reach over 50% in Italy and Spain while [5] reported the NCP rate in chili pepper ranged from 0.26% to 3.56%. This inconsistencies prompted several investigators to suggest that Capsicum should be considered as facultative cross-pollination species [6,7]. This variation depends on the characteristics of the genotypes, the regions, or the environments within the region. Observations on the flower of Serrano pepper verified that NCP in chili pepper occurs on the flower whose pistil is longer than the stamen.

The variation of NCP in crop species can influence method and purpose of crops improvement. Crops which have high levels of NCP are generally bred as hybrid varieties, while crops with low level of NCP are generally bred and released as pure line varieties. The objective of this study was to
determine the NCP rates of some genotypes of chili pepper in Indonesia and the various factors that influence the NCP.

2. Materials and methods

This experiment was conducted from September 2011 until March 2012 at the IPB experimental field Leuwiko and the Plant Breeding Laboratory, Department of Agronomy and Horticuture, Faculty of Agriculture, Bogor Agricultural University. Four genotypes of chili peppers were used in this experiment. IPB C20 genotype, which has purple hypocotyl, was used as pollen source for NCP identification. IPB C2 and IPB C5 genotypes are female and male parents for the hybrid variety of IPB CH3, whereas IPB C120 is an inbred line variety (local variety) of chili pepper were used NCP evaluation.

The experimental field consisted of three blocks of trial in which each of blocks consisted of 9 m² pollen source plot in the central block and 10.5 m² receptor pollen plot in the surrounding the pollen source plot. The pollen source plot was planted by purple hypocotyl genotypes (IPB C20) with spacing radius 0.5m x 0.5m, whereas receptor pollen plot was planted by green hypocotyl genotypes (IPB C2, IPB C5 or IPB C120) with radius 0.5, 1, 1.5, 2.5 and 3.5 m from pollen source plot (figure 1).

![Figure 1. The experimental field.](image)

All seeds were harvested from each plant in pollen receptor plots and were examined for NCP in the next generation. The dominance of purple hypocotyl colors over green hypocotyl colors was utilized to identify NCP. Seeds were sown in transparent plastics box, seeds from NCP produced purple hypocotyl colors after one week. The percentage of NCP was determined by calculating as $T = \frac{H}{P} \times 100\%$, where $H$ is the frequency of seedling with purple hypocotyl color; $P$ is number seeds were sown. In addition, the observations also were made on anther length, carpel length, stigma exertion, and the percentage of successful of artificial cross-pollination. Artificial cross-pollination was done by hand pollination at the first node to twelve nodes on each plant. IPB C2, IPB C5 and IPB C120 genotypes were used as the female parents, whereas IPB C20 genotype was used male parent. The percentage of successful artificial cross-pollination was determined by calculating as $T = \frac{A}{b} \times 100\%$, where $A$ is a success number of artificial pollination; $b$ is number artificial pollination was done.

Data were analyzed using Minitab 17. T-testing was employed when necessary to detect significant differences among means. A probability level of $p < 0.05$ was considered statistically significant. A linear regression correlation test was performed to investigate correlation between percent natural cross-pollination and stigma exertion.
3. Result and discussion

The result showed that there were significant differences in the levels of natural cross-pollination among the genotypes of chili pepper (table 1). This is consistent with [6] that there were differences in the levels of NCP in different types of chili pepper. IPB C2 genotype had the highest percentage NCP with 24.53% while the lower percentage was found on IPB C120 and IPB C5 genotypes with less than 10% (table 1).

Table 1. Percent natural cross-pollination percentages of three genotypes of chili pepper on different planting radius.

| Radius (m) | Genotypes | Means |
|-----------|-----------|-------|
|           | IPB C2    | IPB C5 | IPB C120 |       |
| 0.5       | 22.11     | 6.75   | 6.69      | 11.85 |
| 1         | 25.75     | 3.73   | 15.86     | 15.11 |
| 1.5       | 17.46     | 3.03   | 10.42     | 10.30 |
| 2.5       | 34.20     | 1.08   | 14.86     | 16.71 |
| 3.5       | 23.16     | 6.25   | 0.00      | 9.80  |
| Means     | 24.53\(^a\) | 4.17\(^b\) | 9.57\(^b\) |       |

Values followed by same letters within a row are not significantly different at 5% level of probability.

These results were consistent with the result of field experiment. There were different levels of NCP on different blocks. The highest percentage of NCP was found on IPB C2 block. A total of 60.87% of the plants on IPB C2 block were contaminated by IPB C20 pollen (table 2).

Table 2. Percentages of plants contaminated by IPB C20 pollen.

| Genotypes | Number of plants | Contaminated plants | Percentages of contaminated plants (%) |
|-----------|------------------|---------------------|----------------------------------------|
| IPB C2    | 115              | 70                  | 60.87\(^a\)                            |
| IPB C5    | 120              | 11                  | 9.17\(^c\)                             |
| IPB C120  | 120              | 24                  | 20.00\(^b\)                            |

Values followed by same letters within a column are not significantly different at 5% level of probability.

Percent natural cross-pollination ranged from 9.80% to 16.71% depending on different planting radius. However, there was no significant difference of NCP among different planting radius in this experiment (table 1). It was indicated that wind was not potential factor for chili pepper pollinator. This was reinforced by the result which showed that the pattern of pollen contamination were random and irregular (figure 2). [2] reported that the extension of vegetation, infrequent winds and large population of insect pollinator could be responsible for the high levels of NCP in chili pepper.
Table 3. Anther length, carpel length and stigma exertion in three genotypes of chili pepper.

| Genotype | Anther length (mm) | Carpel length (mm) | Stigma exertion (mm) |
|----------|-------------------|--------------------|---------------------|
| IPB C2   | 2.36c             | 4.54b              | 1.01a               |
| IPB C5   | 3.17a             | 3.32c              | -0.91b              |
| IPB C120 | 2.90b             | 5.50a              | 0.99a               |

Values followed by same letters within a column are not significantly different at 5% level of probability.

Observations on flower morphology showed significant differences on anther length, carpel length and stigma exertion length among the chili pepper genotypes. IPB C5 genotype had the longest anther length whereas IPB C120 genotype had the longest carpel length. The highest stigma exertion was found on IPB C2 and IPB C120 genotypes (table 3). Stigma exertion length was probably one of the factors which responsible for chili pepper NCP. There was significant positive correlation between stigma exertion length and NCP percentages (figure 3). This caused IPB C2 genotype which had the highest stigma exertion length also had the highest NCP percentages while IPB C5 genotypes which had the lowest stigma exertion length also had the lowest NCP percentage. These results were closely parallel with the findings from the previous studies. The positive correlation between stigma exertion length and NCP percentage was also had been reported by [8] and [2] in chili pepper, [9] in *Mimulus ringens*, and [10] in *Datura stramonium*. 

Figure 2. Pattern of natural cross-pollination on three genotypes of chili peppers.

Figure 3. Pattern of natural cross-pollination on three genotypes of chili peppers.
Figure 3. Relationship between percent natural cross-pollination and stigma exertion in chili pepper.

In this experiment, the observations were also made on levels of successful artificial cross-pollination. There were significant differences on the percentage of successful artificial pollination among the different genotypes of chili pepper. IPB C2 genotype had the highest percentage with 50.57% successful artificial pollination, while the lowest percentage was resulted by IPB C5 genotype (table 4). This indicated that IPB C2 genotype had cross ability better than IPB C5 and IPB C120 genotypes. This probably caused IPB C2 genotype which had the same stigma exertion length with IPB C120 genotype, but had the higher NCP percentages than IPB C120 genotype.

Table 4. Artificial-pollination success percentages on three genotypes of chili peppers.

| Genotypes  | Number of pollination | Pollination success | Pollination success percentages (%) |
|------------|-----------------------|---------------------|-------------------------------------|
| IPB C2     | 176                   | 89                  | 50.57<sup>a</sup>                   |
| IPB C5     | 133                   | 19                  | 14.29<sup>c</sup>                  |
| IPB C120   | 193                   | 59                  | 30.57<sup>b</sup>                  |

Values followed by same letters within a column are not significantly different at 5% level of probability

Variation in natural cross-pollination has been attributed to a variety of factors, including: asynchrony in flowering times [11,12]; distance to nearest neighbor [13,14]; pattern of pollinator movement [13,11]; aspects of floral morphology [15,10,16]; and genetic differences in the level of self-fertility [14]. However, stigma exertion length and level of cross ability were two main factors that were responsible on chili peppers NCP in this experiment.

The levels difference of NCP will affect the breeding method of chili peppers. Chili pepper genotypes which had a high level of NCP will likely to behave like cross-pollinated crops. It was also likely to have high heterosis and inbreeding depression so it is very possible to be released as hybrid varieties. [17] reported that hybrid variety IPB CH3 (using IPB C2 as female parent) produced higher of fruit weight and fruit yield per plant than some commercial hybrids whereas [19] reported that IPB C2 genotype had higher heterosis and general combining ability for fruit weight character than the other
genotypes. A good crossability in this genotype will also facilitate the hybrid seed production activities. In contrast to these genotypes, chili pepper genotypes with a low level of NCP will likely to behave like self-pollinated crops. So, it was also likely to have low heterosis and inbreeding depression and it is very possible to be released as inbred line varieties. It was no wonder that IPB C120 genotype (local variety of chili pepper) had long been used as inbred line variety in Indonesia.

References
[1] Allard R W 1960 Principles of plant breeding (New York: Willey) p 254
[2] Campodonico O P 1983 Estimates of natural cross – pollination in serano pepper (Capsicum annuum L.) Capsicum Newsletter 2 pp 106–107
[3] Corella P, Celada V and Csillery C 1986 Natural cross – pollination experiment in Spain 1986. Capsicum Newsletter 5 pp 36–37
[4] Csillery C, Quagliotti L and Rota A 1986 Natural cross – pollination experiment on pepper (Capsicum annuum L.) in Piedmont, Italy, in 1986 Capsicum Newsletter 5 pp 38–39
[5] Kim C G, Kim D I, Kim H J, Park J I, Lee B, Park K W, Jeong S C, Choi K H, An J H, Cho K H, Kim Y S and Kim H M 2009 Assessment of gene flow from genetically modified anthracnose-resistant chili pepper (Capsicum annuum L.) to a conventional crop J Plant Biol 52 pp 251–258
[6] Odland M L and Porter A M 1941. A study of natural cross-pollination (Capsicum frutescens) J. Am. Soc. Hort. Sci 38 pp 585–588
[7] Franceschetti U 1972 Natural cross-pollination in pepper (Capsicum annuum L.) In Belletti P, Nassi MO and Quagliotti L (eds.) Proceeding Eucarpia Meeting on Genetic and Breeding of Capsicum. Turin, Italy. pp 346–353
[8] Murthy N S R and Murthy B S 1962 Natural cross-pollination in chili Andhra Agr. Jour 9 pp 162–165
[9] Karron J D, R T Jackson, N N Tumser and S L Schlicht 1997 Outcrossing rates of individual Mimulus ringens genets are correlated with anther-stigma separation Heredity 79: 365–370
[10] Motten A F and Antonovics J 1992 Determinant of outcrossing rate in a predominant self – fertilizing weed, Datura stramonium (Solanaceae) Amer. Jour. of Bot 79 pp 419–427
[11] Murawski D A and Hamrick J L. 1992 Mating system and phenology of Ceiba pentandra (Bombacaceae) in Central Panama J. Hered 83 401–404.
[12] Boshier D H, Chase M R and Bawa K S 1995 Population genetics of Cordia alliodora (Boraginaceae), a neotropical tree Am. J. Bot. 82 476–483.
[13] Smyth C and Hamrick J L 1984. Variation in estimates of outcrossing in musk thistle population. J. Hered 75 303–307.
[14] Warwick S L and Thompson B K 1989 The mating system in sympatric population of Carduus nutans, Carduus acanthoides and their hybrid swarms. Heredity 63 329–338
[15] Humphreys M O and Gale J S 1974 Variation in wild population of Papaver dubium VII The mating system Heredity 33 33–42
[16] Damgaard C, Couvet D and Loeschecke V 1992 Partial selfing as an optimal mating strategy Heredity 69 289–295
[17] Syukur M, Sujiprihati S, Yunianti R and Kusumah D A 2010 Evaluasi daya hasil hibrida dan daya adaptasinya di empat lokasi dalam dua tahun J. Agron. Indonesia 38 pp 43–51
[18] Arif A B, Sujiprihati S and Syukur M 2012 Pendugaan heterosis dan heterobeltiosis pada enam genotipe cabai menggunakan analisis silang dialel penuh J. Hort 22 103–110

Acknowledgement
 Ministry Research, Technology, and Higher Education Republic of Indonesia and Center for Tropical Horticulture Studies, Bogor, Agricultural University of Indonesia for providing full funding of the experiment and genetic materials.