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## Research Article



## Genetic studies of yield components and pungency in chilli (*Capsicum annum* L.) genotypes through Hayman's approach

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### Abstract

Estimation of genetic parameters using the Hayman approach diallel method was developed in a population formed close to the Hardy-Wienberg equilibrium. The genetic parameter information obtained can be used as the basis for chilli breeding activities, especially for capsaicin content and high productivity. The aim of this study was to obtain information on genetic parameters and gene action that control the capsaicin content and productivity components in chilli using the Hayman approach diallel analysis method. The results showed that all observed characters were controlled by the additive genes action except for the character number of days taken for flowering as indicated by the comparative value of narrow-sense heritability and broad-sense heritability. The distribution of genes in the parents of all observed characters was uneven and all characters were controlled by 1-3 genes. This information is useful for determining the direction of the variety that will be produced in future breeding activity.

**Key words:** Chilli, capsaicin, flowering age, gene action, yield component

### INTRODUCTION

One of the cultivated horticultural plants that have an important role in life is the chilli plant (Kirii *et al.*, 2017). Chilli is widely used because it has pungency due to the capsaicin content in it. In general, fresh chilli contain 0.1-1.0% capsaicin content (Lavorgna *et al.*, 2019). Popelka *et al.* (2017) stated that capsaicin compounds can be found in the seeds, skins and flesh of chillies. The problem that arises is that hot chilli is not matched by high productivity. Plant breeding activities can answer these problems by utilizing existing genetic material which is used as the basis for creating new superior varieties (Srenivas *et al.*, 2020). Syukur *et al.* (2018) also stated that plant breeding is a combination of art and technology to create new superior varieties. One conventional plant breeding technique that is easy to do is artificial hybridization

(Kalve and Tadege, 2017). Ganefianti *et al.* (2019) stated that hybridization aims to combine the genetics of the two parents to produce new varieties by utilizing the heterosis phenomenon. The method of hybridization analysis has been carried out using the diallel analysis method.

The diallel analysis method was carried out to obtain the gene mechanism involved in the early generations (Bisen *et al.*, 2017). Hayman (1954) stated that the diallel analysis method can also predict gene interactions, the influence of dominance and additives, gene distribution and can determine the heritability value of both broad and narrow meanings. The Hayman approach diallel analysis method aims to obtain information on the inheritance of an observed character (Gami *et al.*, 2020).

Information about the inheritance of the traits is valuable for planning and implementing breeding strategies that lead to genetic improvement (Rukundo *et al.*, 2017). Detailed information on the genetic action of selected characters is important if plant breeders carry out their program efficiently with the selection of the appropriate parents and selection methods. In addition, the choice of a competent breeding program depends on a broad knowledge of the working nature of the genes of the traits involved. The genetic control components assist breeders in selecting the desired parents for the crossing as well as the selection methods used. Thus, the aim of this study was to find information about genetic parameters and gene action involved in controlling pungency and yield components in chilli to help create an effective chilli breeding program.

## MATERIALS AND METHODS

This research was conducted at the Leuwikopo Experimental Field, Department of Agronomy and Horticulture, Bogor Agricultural University. Analysis of capsaicin was carried out in the laboratory of the Indonesian Center for Agricultural Postharvest Research and Development, Bogor, West Java. The genetic material used in this study consisted of 36 genotypes consisting of 6 genotypes parental lines of chilli and 30 hybrids. Hybrids resulting from a full diallel cross. The six genotypes of chilli parents were C5, F6074, F9160291, Yuni, Bara, and Giant. This genetic material was a collection of the Genetics and Plant Breeding Laboratory, IPB University. The design used was a Randomized Complete Block Design (RCBD) with three replications and each replication consisting of 20 plants per genotype.

The experimental activity began with seeding activities. Fertilization was done when the seedlings were 2 weeks old using NPK 15:15:15 fertilizer (10 g L<sup>-1</sup> water). Planting was done when the chilli seeds were 30 days old or had reached a plant height of about 15 cm and the number of leaves was 8. Beds measured 1 × 5 m with a distance of 50 cm between beds. The beds were covered with silver black plastic mulch and planting holes were made at a distance of 50 × 50 cm. Maintenance activities were carried out as follows: watering in the morning and evening, fertilizing once a week using NPK fertilizer 15:15:15 (10 g L<sup>-1</sup> water) as much as 250 ml per plant, spraying pesticide once every 2 weeks using a fungicide made from active Mankozeb (2 g L<sup>-1</sup>) and insecticide with active ingredients Prefonofos (2 mL L<sup>-1</sup>). Harvesting was done when the chilli had reached a level of maturity of 75% or at the age of 70 days after planting which was carried out every week for 8 weeks. Observation of the number of days taken for flowering, fruit length, fruit weight, the total amount of fruit per plant, fruit weight per plant, and capsaicin content these variables refer to the Descriptor of Capsicum International Plant Genetic Resources Institute (IPGRI, 1995).

The capsaicin analysis in this research used a modified

HPLC method (Guo *et al.*, 2015). The initial step in the analysis of capsaicin was to dry the sample of chilli using an oven at 50°C for 48 hours. The next step was to measure the water content at 59°C then crush the chillies until smooth. Chilli powder with a weight of 0.5 g<sup>-1</sup> was put into a 50 ml volume test tube to which was added 5 ml of acetone then shaken by hand and using the ultrasonic treatment for 5 minutes at room temperature. The test tube was closed using aluminium foil and heated using a water bath for 8 hours at 80°C. The next step was to cool the sample in the refrigerator overnight at 4°C. The sample was then filtered with Whatman 41 filter paper into a test tube scale and taken 30 mL then ultrasonic for 20 minutes. After that, a part of the solution was taken using a 0.45-micron Syringe Filter and put in a 1.5 ml vial bottle for HPLC. In this study, we used 2 replications per genotype.

HPLC Detector DAD UV-VIS with C18 column (4.6 mm×150 mm, 4 µm). Nagy *et al.* (2018) stated that the C18 column was used for effective partition and quantification of capsaicinoids. The temperature column used in this research was 30°C and 4°C sample temperature with 250 mm and 276-280 nm vv. Fluorescence 1.5 ml min<sup>-1</sup> and injection volume 20 micron. Mobile phase: Acetonitrile : Phosphate Acid 0.1% (40 : 60).

The major capsaicinoids in peppers, capsaicin, and dihydrocapsaicin, were determined by comparison to external reference standards injected under the same conditions (Schmidt *et al.*, 2017). Their identification was based on the retention times measured under identical HPLC conditions, while their quantitative determination in the different pepper samples was carried out using the peak areas. The ratio between these capsaicinoids was calculated by dividing capsaicin and dihydrocapsaicin content by the total capsaicinoids. The capsaicinoid concentrations in the samples are expressed as µg g<sup>-1</sup> pepper.

Capsaicin contents were converted to Scoville Heat Units (SHU) by multiplying the pepper dry weight capsaicin content in g of capsaicin per g of pepper by the coefficient of the heat value for capsaicin; which from literature is 1.6 × 10<sup>7</sup> (Sahid *et al.*, 2020).

The data analysis was performed using ANOVA and Hayman Analysis. ANOVA was performed according to a general linear model (GLM), using the SAS software package. 9.0 and genetic analysis using the Hayman method for yield component and capsaicin content. The analysis of Capsaicin was carried out by analyzing the result of extraction of chilli fruit using HPLC in the order to obtain quantitative data of capsaicin content.

## RESULTS AND DISCUSSION

The results of variance analysis showed a significant difference for the characters viz., the number of days taken for flowering, fruit length, fruit weight, the total

amount of fruit per plant, fruit weight per plant (**Table 1**). The analysis of variance suggested that parents and their  $F_1$ 's exhibited a high amount of genetic variation. Similar results were shown in the study of Salam *et al.* (2017) with a significant difference in the analysis of variance. Estimation of genetic parameters using cross-dialect analysis can be carried out if the quotient of the mean square of the genotype and the mean square of the error for the observed characters has a probability of below 0.01 (Mudhalvan and Kumar, 2020).

The estimated values of the Hayman approach genetic parameters are shown in **Table 2**. It showed that the influence of the environment (E), the additive and dominant components ( $D$ ,  $H_1$ ,  $H_2$ ), the distribution of dominant and recessive genes in the parent ( $h^2$ ,  $H_2/4H_1$ ,  $F$ ,  $Kd/Kr$ ), the number of genes that control a trait ( $h^2 / H_2$ ), and heritability ( $h^2_{ns}$  and  $h^2_{bs}$ ). The results showed that there was no environmental effect found in this study indicated by the value of E which was not significant for all characters. This causes that environmental components do not adequately influence genetic parameters (Cabral *et al.*, 2017).

The number of days taken for flowering showed that the additive component ( $D$ ) was non-significant but significant for the non-additive component ( $H_1$  and  $H_2$ ). In contrast, the character of fruit weight showed a significant result of the additive component ( $D$ ) and the insignificant non-additive component ( $H_1$  and  $H_2$ ). This indicates that the character of the number of days taken for flowering was controlled by a dominant gene action. Whereas fruit weight was controlled by additive genes action seen from the significance of the additive and dominant components. Both additive ( $D$ ) and non-additive components ( $H_1$  and  $H_2$ ) were found significant, indicating the importance of both additive and non-additive gene actions in controlling the expression of fruit length, the total amount of fruit per plant, fruit weight per plant and capsaicin content. In accordance with the research of Ajmal *et al.* (2019) which showed that both additive and non-additive components are significant in the weight character of the fruit crops.

The magnitude of the influence of dominance was shown from the value  $(H_1/D)^{0.5}$ . A value  $(H_1/D)^{0.5}$  that is between 0 and 1 indicates a level of partial dominance, while a value of more than 1 indicates an over-dominance. The

**Table 1. Analysis of variance in chilli genotypes for various traits**

Source of variation	d.f.	Mean squares					
		FA	FL	FW	TFP	FWP	CAP
Replications	2	34.33	1.04	0.46	14.3	4198.42	279927.81
Genotypes	35	55.63**	27.67**	35.5**	5633.74**	52320.58**	544444467.9**
Error	70	3.96	1.1	0.55	6.3	2881.53	400604.03

Note: FA = Number of Days Taken for Flowering, FL = Fruit Length, FW = Fruit Weight, TFP = Total amount of Fruit Plant<sup>-1</sup>, FWP = Fruit Weight Plant<sup>-1</sup>, CAP = Capsaicin content, \*\* Significant at level of 1%.

**Table 2. Estimates of genetic components and other parameters for yield components and capsaicin content in chilli genotypes**

Components	FA	FL	FW	TFP	FWP	CAP
D	9.04 <sup>ns</sup>	14.35**	12.65**	1352.51**	24499.53**	2.97**
F	14.03 <sup>ns</sup>	-2.04 <sup>ns</sup>	-7.92 <sup>ns</sup>	400.47**	-1774.40 <sup>ns</sup>	-1.5**
$H_1$	78.35**	3.51**	10.77 <sup>ns</sup>	801.83**	18630.72**	3.24**
$H_2$	67.13**	2.84**	9.52 <sup>ns</sup>	788.97**	17185.88**	2.72**
$h^2$	131.36**	3.03**	0.24 <sup>ns</sup>	458.69**	8967.48 <sup>ns</sup>	1.96**
E	1.60 <sup>ns</sup>	0.37 <sup>ns</sup>	0.18 <sup>ns</sup>	2.17 <sup>ns</sup>	972.70 <sup>ns</sup>	198626 <sup>ns</sup>
$(H_1/D)^{0.5}$	2.94	0.49	0.92	0.43	0.87	1.04
$H_2/4H_1$	0.21	0.20	0.22	0.24	0.23	0.21
Kd/Kr	1.72	1.00	1.00	1.24	1.00	1
$h^2/H_2$	1.96	1.07	0.03	0.58	0.52	0.72
$h^2_{ns}$	0.14	0.87	0.73	0.91	0.71	0.72
$h^2_{bs}$	0.93	0.96	0.98	0.99	0.95	0.99
$h^2_{ns}/h^2_{bs}$	0.15	0.91	0.75	0.91	0.74	0.73

Note: FA = Number of Days Taken for Flowering, FL = Fruit Length, FW = Fruit Weight, TFP = Total amount of Fruit Plant<sup>-1</sup>, FWP = Fruit Weight Plant<sup>-1</sup>, CAP = Capsaicin content, \*\*Significant at level of 1%, ns = non-significant.

results showed that the capsaicin content and the number of days taken for flowering were over dominant, while other characters (fruit length, fruit weight, total amount of fruit per plant, fruit weight per plant) showed partial dominance. This can be due to the uneven distribution of genes in the parents. The value of gene distribution seen from the  $H_2/4H_1$  ratio was less than 0.25 suggesting the unequal distribution of positive and negative genes. The results showed that all the characters observed had uneven gene distribution. Similar studies showed an uneven distribution of the yield components of tomato (Saleem *et al.*, 2013; Saputra *et al.*, 2014; Pujer and Badiger 2017), rapessed (Tian *et al.*, 2017), and chilli (Fortunato *et al.*, 2015; Pessoa *et al.*, 2019). The number of genes controlling a trait is indicated by the value ( $h^2/H_2$ ). The results showed that all the characters observed were controlled by one gene except for the number of days taken for flowering.

Estimation of broad-sense heritability ( $h^2_{bs}$ ) in all observed characters were included in the high category, namely number of days taken for flowering (0.93), fruit length (0.96), fruit weight (0.98), the total amount of fruit per plant (0.99), fruit weight per plant (0.95), and capsaicin content (0.99). This showed that all the characters observed were controlled by genetic factors (Romana and Farshadfar, 2019). Previous research also showed that the broad-sense heritability value was high in the yield component in chilli (Tembhume and Rao, 2012). In addition, the predicted narrow-sense heritability ( $h^2_{ns}$ ) for all characters was also in the high category except for the number of days taken for flowering (0.14). The heritability value of broad and narrow sense can be used to calculate the additive ratio ( $h^2_{ns}/h^2_{bs}$ ). In line with the values of  $D$ ,  $H_1$ , and  $H_2$  and it showed that the additive ratio for all observed characters was high except for the number of days taken for flowering. This showed that all the characters observed were controlled by the action of additive genes except at the number of days taken for flowering which was controlled by the action of the dominant gene.

It may be inferred and concluded that the capsaicin content and the yield components were controlled by additive genes action. Meanwhile, the number of days taken for flowering is controlled by dominant genes actions. Dominant genes action is directed at the formation of hybrid varieties, while the additive genes action effectively enhances a character. The number of genes that controlled the yield components and capsaicin content was one, while the number of genes that controlled the number of days taken for flowering was three. The distribution of genes in the parents showed unevenness in all observed characters.

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## REFERENCES

- Ajmal, S., Ahsan, A., Rehman, A., Asghar, S., Gulnaz, S., Kanwal, A., Makhdoom, M., Zulkiffal, M., Ghafoor, I. and Ahmad, J. 2019. Estimate of gene action for yield and polygenic yield related traits in bread wheat (*T. aestivum* L.) by using Hayman approach. *Int. J. Biosciences.*, **14** (5): 394-402.
- Bisen, P., Dadheech, A., Namrata., Solanki, A.K.G. and Dhakar, T.R. 2017. Combining ability analysis for yield and quality traits in single cross hybrids of quality protein maize (*Zea mays* L.) using diallel mating design. *J. App. Nat. Sci.*, **9** (3): 1760-1766. [\[Cross Ref\]](#)
- Cabral, N.S.S., Medeiros, A.M., Neves, L.G., Sudré, C.P., Pimenta, S., Coelho, V.J., Serafim, M.E. and Rodrigues, R. 2017. Genotype x environment interaction on experimental hybrids of chili pepper. *Genet. Mol. Res.*, **16** (2). [\[Cross Ref\]](#)
- Fortunato, F.L.G., Rêgo, E.R., Rêgo, M.M., Santos, C.A.P. and Carvalho, M.G. 2015. Heritability and genetic parameters for size-related traits in ornamental pepper (*Capsicum annum* L.). *Acta Horticulturae.*, **1087**: 201-206. [\[Cross Ref\]](#)
- Gami, R.A., Chauhan, B.B. and Patel, R.N. 2020. Hayman's diallel analysis for yield and attributing traits in sesame (*Sesamum indicum* L.). *Elec. J. Pl. Breed.*, **11** (2): 359-366. [\[Cross Ref\]](#)
- Ganefianti, D.W., Arianti, N.N., Sutrawati, M., Saputra, H.E., Fahrurrozi, F. and Herawati. 2019. Superiority test of mixed-cropping models for chili pepper hybrid varieties through participatory plant breeding. *Int. J. Agric. Tech.*, **15** (6): 879-890.
- Guo, C.L., Chen, H.Y., Cui, B.L., Chen, Y.H., Zhou, Y.F., Peng, X.S. and Wang, Q. 2015. Development of a HPLC method for the quantitative determination of capsaicin in collagen sponge. *Int. J. Anal. Chem.*, **1**. [\[Cross Ref\]](#)
- Hayman, B.I. 1954. The theory and analysis of diallel cross. *Genetics.*, **39**: 789-809. [\[Cross Ref\]](#)
- IPGRI. 1995. Descriptors for Capsicum (*Capsicum* spp.). International Plant Genetic Resources Institute. Italia. [\[Cross Ref\]](#)
- Kalve, S. and Tadege, M. 2017. A comprehensive technique for artificial hybridization in chickpea (*Cicer arietinum*). *Plant. Method.*, **13** (52). [\[Cross Ref\]](#)
- Kirii, E., Goto, T., Yoshida, Y., Yasuba, K. and Tanaka, Y. 2017. Non-pungency in a Japanese chili pepper

- landrace (*Capsicum annuum*) is caused by a novel loss-of-function Pun allele. *Hort. J.*, **86** (1): 61-69. [\[Cross Ref\]](#)
- Lavorgna, M., Orlo, E., Nugnes, R., Piscitelli, C., Russo, C. and Isidori, M. 2019. Capsaicin in hot chili peppers: in vitro evaluation of its antiradical, antiproliferative and apoptotic activities. *Plant Foods for Human Nutrition.*, **74**: 164-170. [\[Cross Ref\]](#)
- Mudhalvan, S. and Kumar, N.S. 2020. Genetics of fruit yield and its component characters in okra (*Abelmoschus esculentus* (L.) Moench). *Elec. J. Pl. Breed.*, **11** (1): 124-126. [\[Cross Ref\]](#)
- Nagy, Z., Daoud, H.G., Pék, M. and Helyes, L. 2018. Chromatographic separation of capsaicinoids from spice pepper products on different columns. *J. Liquid Chromatography and Related Technologies.* **41** (11): 740-748. [\[Cross Ref\]](#)
- Pessoa, A.M.S., Rêgo, E.R., Carvalho, M.G., Santos, C.A.P., Mesquita, J.C.P. and Rêgo, M.M. 2019. Genetic inheritance of traits relating to seedling and size in ornamental pepper. *Genet. Mol. Res.*, **18** (1). [\[Cross Ref\]](#)
- Popelka, P., Jevinová, P., Šmejkal, K. and Roba, P. 2017. Determination of capsaicin content and pungency level of different fresh and dried chilli peppers. *Folia Veterinaria.*, **61** (2): 11-16. [\[Cross Ref\]](#)
- Pujer, P. and Badiger, M. 2017. Heterosis and potence ratios for growth, earliness, yield and quality traits in cherry tomato (*Solanum lycopersicum* L. var. Cerasiforme Mill). *Int. J. Chem. Stud.*, **5** (4): 1000-1006.
- Romena, M.H. and Farshadfar, E. 2019. Genetic analysis of stomatal features of some bread wheat cultivars under different water regimes. *J. Anim. Plant Sci.*, **29** (2): 1-13.
- Rukundo, P., Shimelis, H., Mark, L. and Gahakwa, D. 2017. Combining ability, maternal effects, and heritability of drought tolerance, yield and yield components in sweet potato. *Front. Plant. Sci.*, **7** (1981). [\[Cross Ref\]](#)
- Sahid, Z.D., Syukur, M. and Maharijaya, A. 2020. Diversity of capsaicin content, quantitative, and yield components in chili (*Capsicum annuum*) genotypes and their F1 hybrid. *Biodiversitas.*, **21**: 2251-2257. [\[Cross Ref\]](#)
- Salam, J.L., Mehta, N., Tomar, N.S., Saxena, Ritu R., Saxena, Ravi R. and Sarawagi, A.K. 2017. Genetic variability analysis of yield and its components in *Brassica campestris* var. toria. *Elec. J. Pl. Breed.*, **8** (1): 320-323. [\[Cross Ref\]](#)
- Saleem, M.Y., Asghar, M., Iqbal, Q., Rahman, A. and Akram, M. 2013. Diallel analysis of yield and some yield components in tomato (*Solanum lycopersicum* L.). *Pak. J. Bot.*, **45**: 1247-1250.
- Saputra, H.E., Syukur, M. and Aisyah, S.I. 2014. Combining ability and heritability estimation of tomato yield component in full diallel crosses. *J. Agron. Indonesia.*, **42** (3): 203-209.
- Schmidt, A., Fiechter, G., Fritz, E.M. and Mayer, H.K. 2017. Quantitation of capsaicinoids in different chilies from Austria by a novel UHPLC method. *J. Food. Composit. Anal.*, **60**: 32-37. [\[Cross Ref\]](#)
- Srenivas, M., Bhattacharjee, T., Sharangi, A.B., Maurya, P.K., Banerjee, S., Chatterjee, S., Maji, A., Mandal, A.K., Chakraborty, I. and Chattopadhyay, A. 2020. Breeding chili pepper for simultaneous improvement in dry fruit yield, fruit quality and leaf curl virus disease tolerance. *Int. J. Veget. Sci.*, **26** (5): 457-486. [\[Cross Ref\]](#)
- Syukur, M., Sujiprihati, S. and Yunianti, R. 2018. Plant Breeding Technique. Revised Edition. Jakarta (ID): Penebar Swadaya.
- Tembhume, B.V. and Rao, S.K. 2012. Heterosis and combining ability in CMS based hybrid chilli (*Capsicum annuum* L.). *J. Agric. Sci.*, **4** (10). [\[Cross Ref\]](#)
- Tian, H.Y., Channa, S.A. and Hu, S.W. 2017. Relationships between genetik distance, combining ability and heterosis in rapeseed (*Brassica napus* L.). *Euphytica.*, **213**: 1. [\[Cross Ref\]](#)