https://doi.org/10.48048/tis.2024.7709

Breeding for Anthracnose Disease Resistance in Chili Pepper (*Capsicum annum* L.) using Gamma Irradiation

Vichai Puripunyavanich[1,*], Taweepong Na Nan[2], Narisra Suwan[2], Piyanuch Orpong[1], Roppon Picha[1], Lamai Maikaeo[1], Arlee Tamman[3] and Tarntip Bhasabuttra[4]

[1]Nuclear Technology Research and Development Center, Thailand Institute of Nuclear Technology (Public Organization), Nakhon Nayok 26120, Thailand
[2]Nan Agricultural Research and Development Center, Nan 55000, Thailand
[3]Advanced Engineering and Nuclear Technology Center, Thailand Institute of Nuclear Technology (Public Organization), Nakhon Nayok 26120, Thailand
[4]Plant Protection Research and Development Office, Department of Agriculture, Bangkok 10900, Thailand

([*]Corresponding author's e-mail: vichaip@tint.or.th)

Received: 13 November 2023, Revised: 9 December 2023, Accepted: 16 December 2023, Published: 10 June 2024

Abstract

Chili peppers were treated with gamma irradiation to develop anthracnose disease resistance. Seeds of chili peppers were irradiated with gamma rays at 0, 100, 200, 300, 400 and 500 Gy to determine the optimal dose for mutation breeding. The LD_{50} value was calculated to be 264.83 Gy. The 300 Gy gamma-irradiated chili pepper seeds (*Capsicum annuum* L.) were planted in the experiment field at Nan Agricultural Research and Development Center, Thailand for mutation selection. The results showed that the irradiated chili plants not only survived the anthracnose infection but also produced fruits. These fruits were less affected or unaffected by the anthracnose disease compared to the control group (unirradiated) which showed damages on every part of chili plant. The 28 healthy chili pepper plants in M3 generation that showed anthracnose resistant traits were selected as seeds for M4 generation. Two chili pepper lines in M4 generation exhibited the highest resistance to anthracnose. From the pathogenicity test, those 2 chili pepper lines were found to have resistance to anthracnose disease caused by both *Colletotrichum gloeosporioides* and *Colletotrichum capsici*.

Keywords: Anthracnose resistance, Chili pepper, Gamma-ray, Irradiation, Mutation

Introduction

Chili pepper, botanically known as *Capsicum annuum* L., belongs to the genus Capsicum with in the Solanaceae family. The chili pepper, a dicotyledonous plant with many branches, features slender, dark green leaves and insect-pollinated flowers. The color of chili peppers varies from mature green to full red or yellow, depending on the variety. Their fruit exhibits significant variation in shape, pericarp thickness, color and pungency, with pungency ranging from 2,000 to 25,000 Scoville Heat Units (SHU) due to the compound capsaicin. Most cultivated chili peppers belong to one of 2 major species groups: *Capsicum annuum* L. and *Capsicum frutescens* L. Usually, *Capsicum annuum* L. is grown principally for use in commercial chili sauces. While *Capsicum frutescens* L. is one of the most important ingredients in many different cuisines throughout the world as it adds pungency and color to the dishes [1].

In Thailand, chili pepper is one of the most economically important vegetables. Chili can serve as a flavor enhancer in various forms, including fresh chili, dried chili, or ground chili that Thai people have been familiar with for a long time. Thailand, the world's 2^{nd}-largest chili pepper producer, has suitable areas for chili pepper cultivation throughout the year. There is a high demand for chili peppers in both domestic and international markets. In 2022, Thai chili pepper seeds were exported at over 805 million Baht value. They were also imported for more than 60 million Baht for domestic consumption [2]. The value of chili and its derivative exports, mainly processed products such as chili sauce and chili powder, has been steadily increasing [3].

Anthracnose disease, which is caused by fungi (*Colletotrichum spp*.), is one of the major problems for growth and fruit production of chili plants. Severe anthracnose infections can weaken the chili plant, leading to stunted growth, reduced vigor and even death in extreme cases. Anthracnose in chili pepper fruit has adverse effects in chili-growing areas in Thailand, tropical Asia and worldwide [4]. The previous reports have shown that anthracnose infection can cause yield losses of 20 - 80 % in Vietnam [5], 50 % in Malaysia [6] and 10 - 54 % in India [7,8]. In Thailand, the infected chili plant with anthracnose may result in yield losses of up to 70 % and can reach as high as 80 % during severe epidemics [9]. The 2 major Colletotrichum species in Thailand, *C. capsici* and *C. gloeosporioides*, have been reported as the causal agents of chili anthracnose disease [10]. The confirmation of fungal pathogens causing anthracnose symptoms in chili fruits is an important step for understanding and managing the disease. The symptoms of chili pepper infected with these pathogens can occur in various parts including stems, leaves, flowers, or fruits. Some typical symptoms of anthracnose on chili plants include leaf lesion, which may appear as dark spots with irregular shapes, and fruit rot. The infected fruits develop sunken, dark, and often water-soaked lesions with concentric rings of acervuli [11,12]. Such fruits are unattractive and unmarketable. Premature dropping of infected fruits also reduces the overall yield of chili pepper. In addition, this disease can also affect to the quality of fruits by reducing fruit dry weight and the quantities of capsaicin and oleoresin [13,14].

It is important to develop chili pepper to follow domestic and world market demands, characteristics such as shapes, colors, or pest resistance. In Thailand, all chili varieties are susceptible to anthracnose infection, therefore, mutation induction by chemical mutagens or physical mutagens such as ionizing radiations [15] can be used to produce a new genetic germplasm. Radiation can induce beneficial effects on plant characteristics [16] by causing DNA changes through both direct and indirect mechanisms. The direct effect involves direct modifications to the structure and function of DNA molecules induced by radiation energy. Additionally, there are indirect damages caused by free radicals generated through the interaction of water molecules with ionizing radiation [17,18]. Radiation such as X-rays, gamma rays and neutrons can be used, but gamma rays are the most commonly used. For example, the dose of 40 Gy gamma radiation was effectively applied to *Saccharum officinarum* and *Solanum tuberosum* to confer resistance against sugarcane mosaic virus and late blight disease, respectively [19]. Banana mutant lines with resistance to Bunchy Top Virus (BBTV) were developed using gamma irradiation and *in vitro* technique [20]. This research hopes to develop an anthracnose resistant chili pepper that can adapt and survive at the Nan Agricultural Research and Development Center (NNARC) where chili seasoning factories are located nearby. It is expected that a successful breeding of a mutant line will be able to support the economy of the Nan province area. In addition to anthracnose resistance, other characteristics such as large size, dark red color, shrub shape of the plant and upright fruit will be selected in the research program.

Materials and methods

Seed treatment and LD_{50} determination

Chili pepper seeds (*Capsicum annuum* L. 'Sornthong Nan') were obtained from a farmer farm at Tumbol Jomphra, Tha Wangpha District, Nan Province. Chili peppers were irradiated at 0, 100, 200, 300, 400 and 500 Gy of Cobalt-60 gamma radiation (Gamma Chamber 5000, BRIT, India) at Thailand Institute of Nuclear Technology (Public Organization) or TINT, Ongkharak district, Nakhon Nayok province. The irradiated seeds were sown in nursery trays containing peat moss. After 3 weeks of planting, the survival percentage of seedling were recorded. The seedling survival percentages were measured and plotted against the radiation doses. Lethal dose at 50 % or LD_{50} value, which is the dose that causes the death of half the population, was estimated from the graphs between survival percentage and dose of gamma radiation to find the appropriate dose for mutation induction.

Mutation induction and field selection

Chili pepper seeds were irradiated with gamma radiation at 300 Gy which is the approximate LD50 value calculated from a preliminary experiment. The irradiated seeds (M1 seeds) were planted for M1 generation (M1 plants) at NNARC. M2 seeds were harvested from healthy plants and planted in separate rows using ear-to-row system for the subsequent generation. The selection process was started from M2-M5 crop for anthracnose resistance caused by *C. gloeosporioides* or *C. capsici* in the field test at NNARC. The selected M3 - M5 fruits that demonstrated anthracnose resistance in the field were further tested by inoculation with *C. gloeosporioides* and *C. capsici* at TINT. In addition to selecting chili plants that are resistant to anthracnose disease, the other desirable characteristics such as yield, quality of product, plant shape were also selected. The shrub shape of the plant and upright orientation of the fruit are desired because they are easy for harvesting.

Preparation of spore suspension and pathogenicity test

The stock of *C. gloeosporioides* and *C. capsici* were inoculated on potato dextrose agar (PDA) medium and incubated for 7 days at 28 ± 2 °C. After 7 days, 10 mL of phosphate buffer was added to the well-sporulating *C. gloeosporioides* and *C. capsici* plates and a sterile loop or spreader glass was used to scrape and spread to release spores. The suspension was transferred to a sterile conical tube and the suspension was filtered through 4 layers of gauze to remove mycelial fragments. The concentration of filtered suspensions was determined with a haemacytometer and diluted to about 10^6 spore/cm^2. The M4 chili pepper fruits were washed with tap water and decontaminated with 70 % ethanol for surface sterilization. After that, surface-sterilized chili pepper fruits were placed in sterile petri dish glasses. The chili pepper fruit samples were inoculated using the wound/drop inoculation method [21]. The samples were pierced with a sterile needle or core borer in the middle portion of fruit and then 25 µL of suspension was dropped onto the wound. The inoculated chili pepper fruits were incubated at 28 ± 2 °C for 5 days. The results were compared between the irradiated chili fruit samples and the control group (unirradiated).

Results and discussion

Plant sensitivity to radiation varies with its radiation durability, partly controlled by genes which transmit inheritance. Appropriate radiation doses for mutation breeding of various plants have long been studied [22]. LD_{50} was used as the parameter for appropriate radiation dose in this experiment. Seeds of chili peppers were irradiated with gamma rays at 0, 100, 200, 300, 400 and 500 Gy. Their survival percentages and radiation doses were plotted for LD50 determination. From graph between survival percentage (on Y-axis) and gamma dose in Gy unit (on X-axis), LD50 was calculated from the formula of the linear fit function $Y = -0.1876X + 99.683$, and came out to be 264.83 Gy (**Figure 1**) which is near the value the IAEA suggested [23]. However, for practical purpose, 300 Gy dose was used in this study because of the limited accuracy of the gamma irradiator.

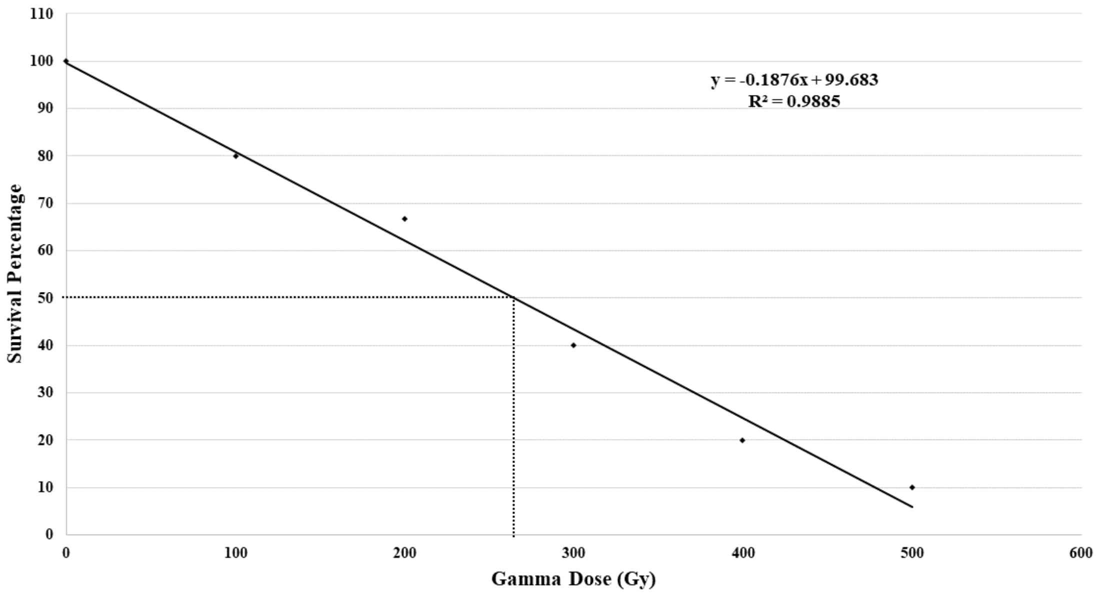

Figure 1 Determination of optimal radiation dose LD_{50} in induced chili seed mutagenesis.

For the mutation induction step, the chili seed were irradiated with 300 Gy of gamma radiation at TINT and planted at NNARC, which is an area that faces anthracnose disease every year. The mutant selection process started in M2 generation. The results showed that the irradiated chili plants not only survived the anthracnose infection but also produced fruits. These fruits showed a resistance trait, which means they were less affected or unaffected by the anthracnose disease compared to the control group (unirradiated) in which there were visible damages on every part of the chili plant. The M2 mutants with anthracnose resistance characteristic were planted and harvested to be used as M3 seeds for screening. In the M3 generation, the selection process was continued. The 28 healthy chili pepper M3 plants that were less affected by anthracnose disease were selected and coded with numbers from 1001 to 1028. These selected plants were cultivated to produce the M4 generation. Among the M4 plants, chili pepper lines with the codes 1009 and 1018 exhibited the highest resistance to anthracnose, showing the least effect from the disease.

Figure 2 Infected chili pepper plants with anthracnose disease (left) non-irradiated chili plant (right) irradiated chili plant with 300 Gy of gamma.

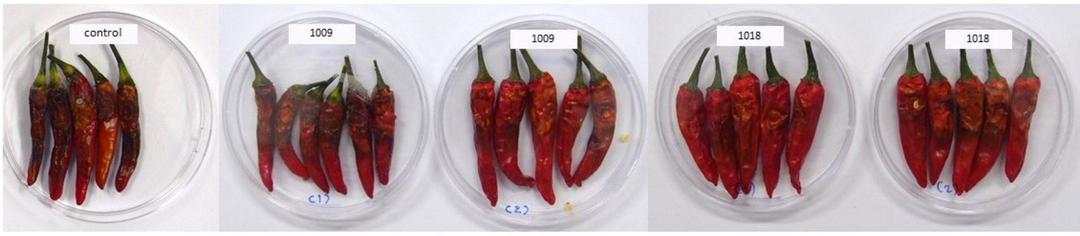

Figure 3 Symptoms on chili fruits after wound/drop inoculation.

The chili pepper mutant having resistance of anthracnose was confirmed using the wound/drop inoculation method which involves transferring a spore suspension onto the surface of sample after it has been pierced. This method has been shown to be useful for selecting resistant varieties of chili [11,21,24]. In this study, the *C. capsici* and *C. gloeosporioides* species were inoculated to chili pepper fruits to confirm the resistance result. The findings revealed that anthracnose-resistant mutants exhibited fewer symptoms. On the other hand, the control samples showed significant effects, with the fruits changing to a dark color and fungal growth observed (**Figure 3**). Therefore, it was possible to select cultivars resistant to anthracnose caused by both fungi, *C. gloeosporioides* and *C. capsici*. This valuable characteristic could result from changes in genes involved in the response to infection due to gamma radiation, influencing disease resistance. The disease resistance can be classified into 2 modes of inheritance [25]. The 1st mode is monogenic disease resistance which involves a single gene, that can be either dominant or recessive. The 2nd mode is polygenic disease resistance that is conferred by many genes. Previous reports have indicated that the anthracnose resistance can be regulated by both a major resistance locus and polygenic resistance and controlled by polygenes with a mainly additive genetic effect [26,27]. However, some other reports have presented evidence for a monogenic dominant inheritance of resistance [28,29].

Besides evaluating resistance to anthracnose, productivity was also examined. The M5 anthracnose resistant chili pepper seeds no. 1009 and no. 1018 were planted and the productivity data were collected. Chili fruits were harvested and the yield data in terms of weight, length, width of fruit and thickness of fruit flesh were collected. The results (**Figure 4**) showed that no. 1009 fruits showed significantly longer and greater weight than no. 1018. While fruits of no. 1018 were slightly wider than those of no. 1009, there was no significant change in fruit width and fruit flesh thickness between 2 lines (**Table 1**). In addition to selecting disease-resistant chili pepper lines, achieving other desirable characteristics such as fruit orientation was valuable. In this study, we found that some plants exhibited the mutant trait of erect chili fruits (**Figure 5**), making them easier to harvest which is especially beneficial for large-scale commercial farming operations where efficiency and productivity are crucial. The fruit tip orientation in chili peppers depends on the chili pepper variety, for example, in *Capsicum annuum* L., the fruit tip can be either pendant or erect, whereas in *C. frutescens*, all fruits are erect [30,31]. The previous studies indicated that chili fruit orientation is regulated by a pair of dominant genes exhibiting inhibitory epistasis and the way fruit orientation is controlled and the number of genes involved vary depending on the genetic background of the materials used for studying the inheritance pattern [32-34]. Moreover, this orientation is inherited as a recessive gene in chili peppers and the up locus on chromosome 12 in *Capsicum annuum* L. is known to control the erect habit [31,35-37]. The effects of radiation on genetic material were planned to study in the further step.

Table 1 Productivity of anthracnose resistance chili pepper no. 1009 and no. 1018.

Mutant line	Fruit weight (g)	Fruit width (mm)	Fruit length (cm)	Fruit flesh thickness (mm)
No. 1009	19.09 ± 6.38^a	10.98 ± 1.93	16.20 ± 3.42^a	1.79 ± 0.46
No. 1018	14.17 ± 3.44^b	12.05 ± 1.53	13.35 ± 2.54^b	1.63 ± 0.22

Note: Different letters show significant differences at $P < 0.05$.

Figure 4 Anthracnose resistance chili pepper fruits of no. 1009 and 1018.

Figure 5 Erect and pendant chili pepper fruits with anthracnose resistance.

The M5 chili pepper seeds of the 2 lines were shared with Tha Wang Pha Community Agricultural Enterprise, which is a farmer enterprise group in Nan province who grew chili pepper as an after-rice crop. The experimental fields of the selected chili pepper lines, which were grown during the anthracnose period in the rainy season around mid-May 2021, were planted both inside the Nan Agricultural Extension and Development Center and in the area plots of Tha Wang Pha Farmers Community Enterprise. In Tha Wang Pha Farmers Community Enterprise, it was observed that both chili pepper lines tended to be disease resistant and produce good yields. Meanwhile, at NNARC, the no.1018 line gave the highest yield of 2,280 kg/rai whereas the no. 1009 line yield was 1,500 kg/rai.

Conclusions

The results show that the anthracnose resistance in chili peppers could be induced by mutation breeding using 300 Gy gamma irradiation. Chili lines obtained in this project exhibited complete resistance to both *C. gloeosporioides* and *C. capsici*. Apart from the anthracnose resistance, we found that some plants exhibited the mutant trait of erect chili fruits which making them easier to harvest. Such characteristics in chili peppers were occasionally found in nature, but gamma irradiation increased the likelihood of discovering these traits. However, selecting upright or point-down chili pepper fruit characteristics might be more complex due to the potential control of this trait by multiple alleles. Further investigations are planned to study the effects of radiation on the chili pepper genotype. Despite the challenges, the aim is to develop chili plants exhibiting 100 % upright fruits, shrub shape, and complete anthracnose resistance to be grown in Thailand in the future.

References

[1] Department of Agriculture and Cooperation, Ministry of Agriculture of India. *Post harvest profile of chilli*. Department of Agriculture and Cooperation, New Delhi, India, 2009.
[2] Office of Agricultural Regulation, Available at: https://www.doa.go.th/ard/, accessed November 2023.
[3] Office of the Permanent Secretary Ministry of Commerce, Available at: https://tradereport.moc.go.th/, accessed October 2023.
[4] K Ratanacherdchai, HK Wang, FC Lin and K Soytong. RAPD analysis of *Colletotrichum* species causing chili anthracnose disease in Thailand. *J. Agr. Tech.* 2017; **3**, 211-9.
[5] LD Don, TT Van, VYTT Phuong and PT Kieu. *Colletotrichum spp.* attacking on chilli pepper growing in Vietnam. Country report. *In*: Proceedings of the 1st International Symposium on Chilli Anthracnose, Seoul, South Korea. 2007, p. 24.
[6] M Sariah. Incidence of *Colletotrichum spp.* on chili in Malaysia and pathogenicity of *C. gloeosporioides*. *Biotrop Spec. Publ*. 1994; **54**, 103-20.
[7] KK Lakshmesha, N Lakshmidevi and SA Mallikarjuna. Changes in pectinase and cellulase activity of *Colletotrichum capsici* mutants and their effect on anthracnose disease on capsicum fruit. *Arch. Phytopathology Plant Protect.* 2005; **38**, 267-79.
[8] N Ramachandran and K Rathnamma. *Colletotrichum acutatum* - A new addition to the species of chilli anthracnose pathogen in India. *In*: Proceedings of the Annual Meeting and Symposium of Indian Phytopathological Society, Kasaragod, India. 2006, p. 27-8.
[9] S Poonpolgul and S Kumphai. Chilli pepper anthracnose in Thailand. *In*: Proceedings of the 1st International Symposium on Chilli Anthracnose, Seoul, South Korea. 2007, p. 23.
[10] O Mongkolporn, P Montri, T Supakaew and PWJ Taylor. Differential reactions on mature green and ripe chili fruit infected by three *Colletotrichum* spp. *Plant Dis.* 2010; **94**, 306-10.
[11] PP Than, R Jeewon, KD Hyde, S Pongsupasamit, O Mongkolporn and PWJ Taylor. Characterization and pathogenicity of Colletotrichum species associated with anthracnose on chilli (*Capsicum spp.*) in Thailand. *Plant Pathol*. 2008; **57**, 562-72.
[12] R Ridzuan, MY Rafii, SI Ismail, MM Yusoff, G Miah and M Usman. Breeding for anthracnose disease resistance in chili: Progress and prospects. *Int. J. Mol. Sci*. 2018; **19**, 3122.
[13] P Azad. Fate and role of chemical constituents of chilli fruits during infection with *Colletotrichum capsici*. *Indian Phytopathology* 1991; **44**, 129-31.
[14] DS Mistry, LP Sharma and ST Patel. Bio-chemical parameter of chilli fruits as influences by *Colletotrichum capsici* (Sydow) Butler & Bisby infection. *Karnataka J. Agr. Sci.* 2010; **21**, 586-7.
[15] N Klaipongpan. *Plant breeding technique* (*in Thai*). Kasetsart University Press, Bangkok, Thailand, 2003.
[16] S Lamseejan. *Mutation of the plant* (*in Thai*). Faculty of Science, Kasetsart University, Bangkok, Thailand, 1997.
[17] ME Lomax, LK Folkes and P O'Neill. Biological consequences of radiation-induced DNA damage: Relevance to radiotherapy. *Clin. Oncol.* 2013; **25**, 578-85.
[18] L Ma, F Kong, K Sun, T Wang and T Guo. From classical radiation to modern radiation: Past, present, and future of radiation mutation breeding. *Front. Publ. Health* 2021; **9**, 768071.

[19] A Bhoi, B Yadu, J Chandra and S Keshavkant. Mutagenesis: A coherent technique to develop biotic stress resistant plants. *Plant Stress* 2022; **3**, 100053.
[20] OP Damasco, FMD Cueva, JC Descalsota and RRP Tayobong. Gamma radiation and *in vitro* induced Banana Bunchy Top Virus (BBTV) resistant mutant lines of banana cv 'Lakatan' (*Musa* sp., AA). *Philippine J. Sci*. 2019; **149**, 159-73.
[21] Q Lin, C Kanchana-udomkarn, T Jaunet and O Mongkolporn. Genetic analysis of resistance to pepper anthracnose caused by *Colletotrichum capsici*. *Thai J. Agr. Sci*. 2002; **35**, 259-64.
[22] Food and Agriculture Organization of the United Nations and International Atomic Energy Agency. *Manual on mutation breeding*. 2nd eds. International Atomic Energy Agency, Vienna, Austria, 1977.
[23] Food and Agriculture Organization of the United Nations and International Atomic Energy Agency. *Manual on mutation breeding*. 3rd eds. Food and Agriculture Organization of the United Nations, Rome, Italy, 2018.
[24] S Phoulivong, EHC McKenzie and KD Hyde. Cross infection of *Colletotrichum* species; a case study with tropical fruits. *Curr. Res. Environ. Appl. Mycol.* 2012; **2**, 99-111.
[25] AM Ansari. Molecular markers in vegetable improvement. *Horticultural Biotechnol. Res.* 2015; **1**, 5-10.
[26] N Ahmed, SK Dey and JS Hundal. Inheritance of resistance to anthracnose in chilli. *Indian Phytopathology* 1991; **44**, 402-3.
[27] SW Lin, PA Gniffke and TC Wang. Inheritance of resistance to pepper anthracnose by *Colletotrichum acutatum*. *Acta Horticulture* 2007; **760**, 329-34.
[28] Q Lin, CK Udomkarn, T Jaunet and O Mongkolporn. Inheritance of resistance to pepper anthracnose caused by *Colletotrichum capsici*. *Capsicum Eggplant Newslett.* 2002; **21**, 85-8.
[29] SH Kim, JB Yoon, JW Do and HG Park. Resistance to anthracnose caused by *Colletotrichum acutatum* in chili pepper (*Capsicum annuum* L.). *J. Crop Sci. Biotechnol*. 2007; **10**, 277-80.
[30] K Iwai and T Watanabe. *Red peppers science of pungency*. Saiwaishobo, Tokyo, Japan, 2000.
[31] HR Lee, MC Cho, HJ Kim, SW Park and BD Kim. Marker development for erect versus pendant-orientated fruit in *Capsicum annuum* L. *Mol. Cells* 2008; **26**, 548-53.
[32] TR Gopalakrishnan, R Gopalkrishnan and KV Peter. Inheritance of clusterness and fruit orientation in chilli (*Capsicum annuum* L.). *Indian J. Genet*. *Plant Breed.* 1989; **49**, 219-22.
[33] KPM Dhamayanthi and VRK Reddy. Inheritance of clustered fruit characters and upright fruit orientation in chilli (*Capsicum annuum* L.). *J. Spices Aromat. Crops* 2001; **10**, 41-3.
[34] C Anilkumar, AM Rao, S Ramesh, B Bhavani and Pranesh. Inheritance of fruiting habit traits in chilli (*Capsicum annuum* L.). *Curr. Sci*. 2020; **118**, 1598-602.
[35] LF Lippert, BO Bergh and PG Smith. Gene list for the pepper. *J. Hered*. 1965; **56**, 30-4.
[36] J Cheng, C Qin, X Tang, H Zhou, Y Hu, Z Zhao, J Cui, B Li, Z Wu, J Yu and K Hu. Development of a SNP array and its application to genetic mapping and diversity assessment in pepper (*Capsicum* spp.). *Sci. Rep*. 2016; **6**, 33293.
[37] AM Solomon, TG Kim, K Han, HY Lee, A Patil, MI Siddique, J Ahn and BC Kang. Fine mapping and candidate gene identification for the *CapUp* locus controlling fruit orientation in pepper (*Capsicum spp.*). *Front. Plant Sci*. 2021; **12**, 675474.