MUTATION BREEDING FOR ANTHRACNOSE RESIATANCE CAUSED BY *Colletotrichum capsici* IN CHILI

VICHAI PURIPINYAVANICH[1], PRARTANA KEWSUWAN[2], NARISRA SUWAN[3]
PENJAN SUTTHANUKUL[4] AND TARNTIP BHASABUTTRA[5]
[1]*Thailand Institute of Nuclear Technology, Nakhon Nayok, Thailand*
E-mail: vichaip@tint.or.th
[2]*Thailand Institute of Nuclear Technology, Nakhon Nayok, Thailand*
E-mail: prartanakewsuwan@gmail.com
[3]*Nan Agricultural Research and Development Center, Nan, Thailand*
E-mail:ungagricmu@gmail.com
[4]*Sukhothai Horticultural Research Center, Sukhothai, Thailand*
E-mail:penchan40@gmail.com
[5]*Plant Protection Research and Development Office, Department of Agriculture, Bangkok, Thailand*
E-mail:tip2511@gmail.com

Abstract: In Thailand, there are three major species of *Colletotrichum spp*. which caused anthracnose disease to chili product; *Colletotrichum gloeosporioides*, *C. capsici* and *C. acutatum*. In 2015, the researchers from Thailand Institute of Nuclear Technology reported a first success of radiation-induced mutation breeding for anthracnose resistance which caused by *C. gloeosporioides* in Sukhothai. Anywhere there was no anthracnose disease caused by *C. capsici* or *C. acutatum* in this area. Therefore, breeding program for anthracnose resistance caused by *C. capsici* was moved to Nan, a mountainous area where chili plants are often infected with *C. capsici*. M_1 seed of irradiated CA-1131, hybrid chili (3228 x CA1331), pure CA1131 and the two seclected CA1131 (anthracnose resistance causing by *C. gloeosporioides*) lines were planted in Nan agricultural and development Research Center in rainy season of 2016-2018. The results revealed that anthracnose disease caused by complex *C. gloeosporioides* and *C. capsici*. After three years, anthracnose resistance lines were investigated in 2018. Twenty-eight anthracnose resistance chili plants in field experiment were selected after anthracnose appearance in Nan province in M_3 generation. Ten samples of resistance chili fruits per plant were inoculated with *C. capsici* and the result showed that the chili fruit No.4 was show a completely fresh without any infection after inoculation.

Keywords: *chili, anthracnose, colletotrichum capsici, mutation*

1. INTRODUCTION

Mutation breeding is one of the most effective strategy for trait selection since the release of the first radiation induced variety [1,2], a light green mutant of tobacco released in Indonesia in the mid-1930s. Easy targets for plant mutation breeding are annual, inbred, seed propagated crops. Seeds are ideal for mutation induction. It's mean mutant generations can be produced quickly and desirable mutant line can be developed into varieties rapidly [3]. Several cultivars derived from direct utilization of induced mutants have shown that, for example, short dwarf, earliness and resistance to certain diseases can be introduced in otherwise well-adapted varieties without significantly altering their other attributes. However, one should watch for pleiotropic effects of mutated genes and other unwanted induced mutation. If undesirable characters are observed, it is advisable to cross the mutant back to the parent line and select for individual containing the desired mutation that are free of undesirable changes [4]. Mutagens mainly divide to two type: physical mutagens and chemical mutagens. Physical mutagens comprise all nuclear radiations and sources of

radioactivity including ultraviolet light, x-ray, gamma ray, beta and alpha particles or electron beam. Several types of ionizing radiation are available for plant mutation induction. However, there are several differences among ionizing radiations regarding the energy deployed the penetrating capability also. In Thailand, there was first pioneer paper mentioned to preliminary test of chili gamma irradiation dose rate in 2007. It indicated 400 to 600 Gy of gamma irradiation were appropriated for chili seed irradiation. Irradiated seed was planted in experiment field for six generations. Shrub chilies with thick leaves which resisted to piercing sucking insects were investigated [5].

Chili which botanically known as *Capsicum annuum* L.; *Capsicum frutescens* L., also called red pepper belongs to the genus capsicum, under the solanaceae family. Chili is considered as one of the most important commercial spice crops and is widely used universal spice. It is believed to have originated in South America. Chili is integral and the most important ingredient in many different cuisines around the world as it adds pungency, taste, flavor and color to the dishes [6]. Chili anthracnose disease is not only a serious problem in Asia but also a major constraint to the chili production in the tropics and subtropics worldwide [7]. Anthracnose is an important disease of chili affecting both fruit and seed quality. In Thailand, chili was exported as fresh fruit and chili products with more than 40 million dollars per annual. When chili was severely infected, yield losses can reach up to 50%. Recent years, conventional management of anthracnose in chili has relied on aggressive chemical approaches that are vulnerable to the development of fungicide resistance and leave chemical residues harmful to human health and the environment [8]. The disease is more severe in hot and humid atmosphere area such as subcontinent of India and South East Asia countries, Africa and Latin America. The infected leaves and fruits have small or large lesions, or purplish or brown patches without the formation of definite lesions. Symptoms are seen on leaf, stem and fruit with small, circular spots on the skin of the fruit and expanded in direction of long axis of the fruit and the fruits with many spots drop off prematurely resulting in heavy loss of yield. Fungus may also attack the fruit stalk and spread along the stem causing dieback symptoms. The stems and petioles have girdles and turn yellow, causing dieback and shrinking. As the fruit ripens, its susceptibility to infection increases. The lesions on the fruit are circular and may reach 3 cm in diameter or bigger on the larger fruit. The concentric rings at the center of the lesion may be tan or orange to black. Initial infections are undefined tanned colored lesions that may appear in a matter of a few days after infection.

In Thailand, there are three major species of *Colletotrichum spp.* which caused damage to chili product; *Colletotrichum gloeosporioides*, *Colletotrichum capsici* and *Colletotrichum acutatum*. In 2015, the researchers from Thailand Institute of Nuclear Technology co-operated with Sukhothai Horticultural Research Center, reported a success of mutation breeding for anthracnose resistance in chili which caused by *C. gloeosporioides* in Sukhothai and nearby area [9]. However, there was no any anthracnose disease caused by *C. capsici* species ever been found in Sukhothai province [10].

Therefore, this project aimed to pool and investigate anthracnose resistance in chili caused by *C. capsici* and/or *C. acutatum* in Nan Agricultural Research and Development Center.

2. RESEARCH SUBJECTS AND METHODOLOGY

2.1. Research subjects

From the first phase of mutation breeding for anthracnose resistance lines in Sukhothai province, we got anthracnose resistance caused by *C. gloeosporioides* in hybrid chili (3228 x CA1331) and radiation induced CA1131 line, so we started with these hybrid chili seed from Sukhothai Horticultural Research Center in first part. The second and the

third part, we used the two seclected CA1131 lines from Sukhothai. The fourth part, we used pure CA1131 line which was permitted from AVRDC (Asian Vegetable Resarch and Development Centre, Kasetsart university, Kampangsaen campus). And the last part, we used repeated CA1131- 300 Gy gamma irradiation. Our objective in this seceond phase aimed to characterize anthracnose resistance chili caused by both *C. gloeosporioides* and *C. capsici*. In addition, shrub shape chili, numerous lateral branch with high yield, point up chili fruit instead the normal point down fruit which convenience for harvesting characters were investigated too.

Methodology

1. Irradiated CA1131 chili seed at 300 Gy using gamma ray [10] were planted for M_1 generation in Nan Agricultural Research and Development Center. The healthy M_1 chili plants were selected for receiving M_2 seeds, then planted by ear to row system in M_2-M_3 generation.
2. Hybrid chili (3228 x CA1331), pure CA1131 and the two seclected CA1131 (anthracnose resistance caused by *C. gloeosporioides* character) lines were synchronized planting with M_1-M_3 in Nan Agricultural Research and Development Center.
3. Selection in M_3 crop for anthracnose resistance which caused by *C. gloeosporioides* or *C. capsici* or *C. acutatum* or complex in Nan Agricultural Research and Development Center.
4. *C. capsici* inoculation in selected M_3 fruits which showed anthracnose resistance in the field experiment at Thailand Institute of Nuclear Technology laboratory.
5. Find out for shrub shape chili plants and point up chili fruit character.

2.2. Results

M_1 seed of irradiated CA-1131, hybrid chili (3228 x CA1331), pure CA1131 and the two seclected CA1131 (anthracnose resistance caused by *C. gloeosporioides*) lines were planted in Nan agricultural and development Research Center in the rainy season of 2016. In this year round, anthracnose caused by *C. gloeosporioides* was found. Hybrid chili (3228 x CA1331), and the two seclected CA1131 (anthracnose resistance caused by *C. gloeosporioides*) lines from Sukhothai had been still shown the best anthracnose resistance caused by *C. gloeosporioides* performance. The pure CA1131 and the repeated irradiated CA1131 line were also showed the satisfied resistance. Unfortunately, anthracnose disease caused by *C. capsici* or *C. acutatum* was not found in that year. M_2 seed of every healthy lines in experiment field were collected for next generation. M_2 crops were planted in 2017 but the weather in this year was rather dried and drought until no anthracnose prevalence in all six months period crop. However, healthy and rather shrub shape chili plants with high yield and point up fruits were occured in this crop (fig.1). M_3 seed from interested plants were collected for next crop. Later, around four hundred of chili trees were planted in ear to row system in M_3 crop for anthracnose resistance line investigation in 2018. Twenty-eight chili anthracnose resistance plants in field experiment were selected after anthracnose appearance in Nan province in M_3 generation. Anthracnose disease in this year round was caused by complex *C. gloeosporioides* and *C. capsici* without *C. acutatum*. Ten samples of resistance chili fruits per each from selected twenty eight shrub shape plants in field experiment were inoculated with 10^7 spores/cm^3 concentration of *C. capsici* in Thailand Institute of Nuclear Technology laboratory. The result showed that the no.4 sample fruits which from the CA1131 R2T4 chili plant (table1, fig.2) were completely resisted to anthracnose after inoculation. And some lines show partial resistance (fig.3).

Table 1 Selected shrub shape chili lines with their qualification

Line no.	Chili line	20 fruits weight (g)	% infection	Total weight (g/plant)	Fruit length (mm)	Fruit width (mm)
1	CA1331 R1 T2	21.1	0.50	806	23.4	7.8
2	CA1331 R2 T1	22.8	4.80	967	30.0	8.6
3	CA1331 R2 T3	31.4	3.16	1646	41.3	7.6
4	CA1331 R2 T4	25.7	5.28	1987	38.4	8.2
5	CA1331 R3 T1	21.4	2.49	1115	32.9	7.6
6	CA1331 R3 T2	28.1	2.67	884	42.0	7.8
7	CA1331 R4 T1	31.4	2.60	846	43.9	7.9
8	CA1331 R4 T2	21.4	4.25	1495	30.1	7.6
9	CA1331C1 R1 T1	20.9	6.78	1059	30.8	7.6
10	CA1331C1 R2 T2	35.8	4.34	1034	28.9	7.4
11	CA1331C1 R2 T3	21.5	0.81	815	30.0	7.8
12	CA1331C1 R3 T1	23.1	2.54	976	31.9	7.9
13	CA1331C1 R3 T2	23.1	2.54	1097	33.3	7.5
14	CA1331C1 R4 T1	21.5	0.58	870	27.9	7.6
15	CA1331C2 R1 T4	22.1	1.59	938	31.3	7.3
16	CA1331C2 R1 T5	21.3	1.93	1188	29.8	7.7
17	CA1331C2 R2 T2	20.1	2.56	882	30.0	7.2
18	CA1331C2 R2 T3	22.4	6.32	863	31.9	7.3
19	CA1331C2 R4 T1	21.4	2.39	967	27.2	7.9
20	CA1331 GAMMA R1 T5	20.0	1.64	907	28.4	7.4
21	CA1331 GAMMA R2 T4	19.7	2.05	1077	28.1	7.4
22	CA1331 GAMMA R3 T2	21.0	0.35	788	33.3	7.1
23	CA1331 GAMMA R4 T1	20.1	1.17	662	30.3	7.0
24	32-2-8xCA1331 R1 T1	29.2	4.51	1579	34.5	8.6
25	32-2-8xCA1331 R1 T6	29.5	11.03	1383	39.4	8.2
26	32-2-8xCA1331 R2 T4	33.8	3.76	796	39.7	8.4
27	32-2-8xCA1331 R2 T5	29.5	1.11	1315	34.8	8.6
28	32-2-8xCA1331 R3 T3	29.8	3.6	1230	33.4	9.1

Fig.1 Point up (A) and point down (B) chili fruits which show anthracnose resistance in field experiment compare with the mixed point up and point down fruits wild type (C)

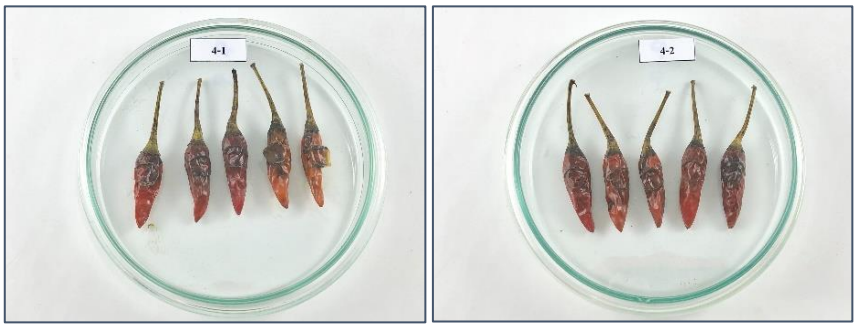

Fig.2 Chili fruits samples no.4 from the CA1131 R2T4 chili plant which showed a completely resistance against anthracnose resistance after *C. capsici* inoculation

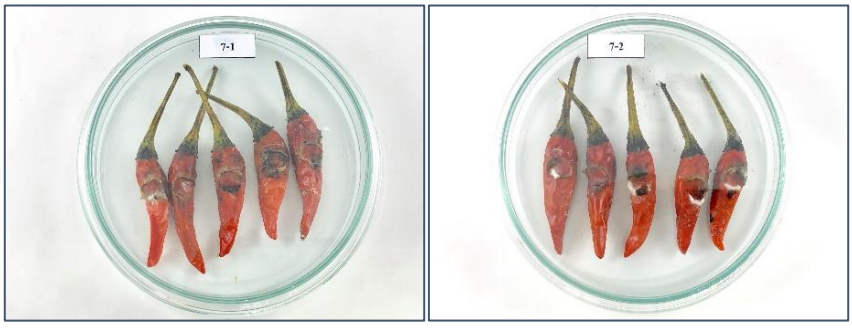

Fig.3 Chili fruit samples no.7 from the CA1131 R4T1 chili plant which showed partial resistance against anthracnose resistance after *C. capsici* inoculation

2.3. Discussion

In general breeding, dominant resistance is more useful than recessive resistance because it will be manifested in F_1 hybrids even if only one parent has the allele additionally [11]. The proportion of F_1 hybrids in worldwide market is growing. Producing F_1 varieties using recessive resistance sources requires much time and effort. However, recessive

resistance is more durable than dominant resistance. This information can benefit chili breeding programs in the production of anthracnose-resistant F_1 varieties.

Our results showed that anthracnose resistance trait could be induced and screened by radiation mutation breeding. Anthracnose resistance chili lines which collected in this project were not known as a dominant mutant or recessive mutant. The No.4 chili line was complete resistance to both *Colletotrichum gloeosporioides* and *C. capsici* but its resistance to anthracnose caused by *C. acutatum* has not investigated yet. Therefore, the project of mutation induction for anthracnose resistance in chili by electron beam irradiation has to carry on base on this research in next phase.

In addition to the main anthracnose resistance objective, shrub shape chili and numerous lateral branch with high yield and point up chili fruit instead the normal point down fruit which convenience for harvesting were also investigated. Usually shrub shape chili and numerous lateral branch character was occasionally found in CA1131 variety but irradiated by electron beam in this chili was increase the probability to find out this character. However, point up or point down chili fruit character selection may be more complicate. Normally we could found point up or point down chili fruit in same variety, sometime we could found point up and point down chili fruit in same tree. This character may be controlled by several alleles. Anywhere, we hope that we can find out 100% point up chili fruit with shrub shape chili and anthracnose resistance to the three major species of *Colletotrichum spp.* chili varieties in Thailand in the future.

3. CONCLUSION

Breeding program for anthracnose resistance caused by *Colletotrichum capsici* was carried out in Nan province, northern region of Thailand. Five chili lines were planted in Nan agricultural and development Research Center in 2016-2018. After three years, anthracnose resistance caused by *C. capsici* lines were screened in 2018. Twenty-eight chili anthracnose resistance plants in field experiment were selected in M_3 generation. Ten samples of resistance chili fruits per plant were inoculated by 10^7 spores/cm^3 *C. capsici* and found chili fruit samples no.4 showed complete resistance. In addition, shrub shape and numerous lateral branch chili and point up chili fruit were also selected.

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