Full Length Research Paper

Cross-species amplification of 349 melon (*Cucumis melo* L.) microsatellites in gherkin (*Cucumis anguria* L.)

Yuichi Matsumoto¹*, Nobuyoshi Watanabe² and Tsutomu Kuboyama²

¹United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan. ²College of Agriculture, Ibaraki University, Inashiki, Ibaraki 300-0393, Japan.

Accepted 20 January, 2012

Gherkin (*Cucumis anguria* L.), also known as West Indian gherkin, burr gherkin, and maxixe, is mainly cultivated and consumed in India, Brazil, and the United States. Marker-assisted selection (MAS) is a highly desirable tool for gherkin breeding, because gherkin cultivation generally requires time, labor, and space. However, few DNA markers for gherkin have been reported. Cross-species amplification of 349 melon (*Cucumis melo* L.) microsatellite primer pairs was tested on three gherkin accessions. After polymerase chain reaction optimization, 149 (42.7%) microsatellite primer pairs successfully amplified all accessions. Of the amplified primer pairs, 41 (27.5%), 64 (43.0%), and 70 (47%) showed polymorphisms between the accessions PI 147065 and PI 320052, PI 147065 and PI 364475, and PI 320052 and PI 364475, respectively. The remaining 206 primer pairs did not amplify any of the three accessions. In the polymorphic primer pairs, the correlation coefficient between repeat number and polymorphic information content values was low; therefore, it seemed unnecessary to consider it for application of repeat numbers in gherkins. Current polymorphic microsatellite primer pairs would be useful for genetic analysis, landmarks in linkage studies, studying genome structure, MAS and evolutionary ecology of Cucurbitaceae.

Key words: West Indian gherkin, Cucumis spp., simple sequence repeat (SSR), polymorphism, breeding.

INTRODUCTION

Gherkin (*Cucumis anguria* L.) is also known as West Indian gherkin, burr gherkin, or maxixe and, like melon, belongs to the subgenus *Melo* (*Cucumis melo* L.) (Kirkbride, 1993). It is mainly cultivated and consumed in India, Brazil, and the United States (Mangan et al., 2008). The fruits are either boiled, fried, stewed, or used fresh in salads and are a valuable source of vitamins and minerals (Resende, 1998). Recent studies have reported the susceptibility of gherkin plants to pathogens causing diseases in melon or cucumber (*C. sativus* L.) plants, such as mosaic virus (Srinivasulu et al., 2010), streak virus (Krishnareddy et al., 2003), powdery mildew (Lebeda, 1984), and fusarium wilt (Matsumoto et al., 2011). Although new cultivars have been developed by

cross breeding (Modolo and Costa, 2004), none of these cultivars have resistance against the previous mentioned pathogens. For suitable cultivation, it is necessary to breed cultivars resistant to these diseases. Molecular genetics technologies such as marker-assisted selection (MAS) could be highly desirable tools for gherkin breeding, because gherkin cultivation generally requires time, labor, and space. However, only few DNA markers are available for gherkins (Chung et al., 2006; Levi et al., 2005); these include loci for producing bitter fruit: *Bt* (Koch and Costa, 1991), loci that control fruit spaniness: *S* and *P* (Koch and Costa, 1991), and loci that controls the resistance to Cucumber green mottle mosaic virus: *Cgm* (den Nijs, 1982).

Microsatellite DNA markers are the genetic markers of choice in mammalian and many plant species because of their abundance, high degree of polymorphism, and suitability for automation (Weber and May, 1989). Microsatellite markers have several advantages over other

^{*}Corresponding author. E-mail: yu-matsumoto@agri.pref. ibaraki.jp. Tel: +81 299 45 8330. Fax: +81 299 45 8351.

molecular markers, and hence, are more reliable for DNA fingerprinting. They show co-dominant inheritance, have large number of alleles per locus, and are abundant in genomes. In addition, the use of microsatellites is based on polymerase chain reaction (PCR) method, which is a simple technique and requires only small amount of DNA. However, novel microsatellites often have to be isolated before they can be used for each species, which generally demands considerable time and high costs. Recently, it has been widely accepted that microsatellites isolated from the source genome can be transferred to different individuals of the same species or the same genus (Barbará et al., 2007). For example, cross-species amplification is prevalent in Brassica (Szewc-McFadden et al., 1996), Actinidia (Weising et al., 1996), and Prunus (Downey and lezzoni, 2000).

In the family Cucurbitaceae, the cross-species application was mainly reported in major crops such as melon, cucumber, pumpkin (Cucurbita maxima Duchesne), and watermelon (Citrullus lanatus [Thunb.] Matsum and Nakai) (Danin-Poleg et al., 2000; Chiba et al., 2003; Fukino et al., 2007) but not in gherkin. To date, many microsatellite linkage maps have been constructed and reported in melons (Danin-Poleg et al., 2001; Joobeur et al., 2004; Ritschel et al., 2004; Gonzalo et al., 2005; Fukino et al., 2007). If melon microsatellite markers are useful and commonly mapped in gherkins, they would be also suitable as anchor markers for studies on synteny. In this study, we attempted to transfer microsatellite primers derived from melon to gherkins, and assess the amplifycation and polymorphism between three accessions of gherkin.

MATERIALS AND METHODS

Plant materials and microsatellite markers

Three accessions of gherkin, PI 147065, PI 320052, and PI 364475, collected from Brazil, Ethiopia, and South Africa, respectively, were used in this study. These seeds were obtained from the Germplasm Resources Laboratory (USDA, Agricultural Research Service, Beltsville, Maryland, USA). A total of 349 microsatellite primers derived from melon (Danin-Poleg et al., 2001; Chiba et al., 2003; Joobeur et al., 2004; Ritschel et al., 2004; Gonzalo et al., 2005; Fukino et al., 2007) were used for PCR amplification in gherkins (Tables 1 and 2).

Polymerase chain reaction (PCR) amplification

Total genomic DNA was extracted from leaves of each plant using DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA). The extracted DNA was diluted to a final concentration of 30 to 50 ng μ L⁻¹ prior to PCR. Amplifications were performed in 10 μ L volumes containing 30 to 50 ng of genomic DNA, 0.25 μ M of each primer, and 5 μ L of 2× Go Taq PCR MasterMix (Promega Co., Madison, WI, USA) using the following conditions: 94°C for 3 min followed by 35 cycles of 30 s at 94°C, 1 min at 55°C, 1 min at 72°C, and a final extension step of 5 min at 72°C. The PCR products were labeled using post-PCR labeling (Kukita and Hayashi, 2002) and separated and detected using an ABI prism 3100/xl genetic analyzer (Applied

Biosystems Inc., Foster City, CA, USA) with each capillary containing 1 μ L of PCR product, 0.1 μ L GeneScan-500 LIZ Size Standard (Applied Biosystems), and 8.9 μ L of HiDi formamide (Applied Biosystems) that was denatured at 95°C for 5 min. The sizes of the fragments were estimated using the Gene Scan Software (Applied Biosystems).

Calculation of polymorphic information content (PIC) values

The information values of microsatellites were determined using their PIC values, which were calculated using version 1.0 of the Marker Tool Kit (Fujii et al., 2008). The PIC values were also used for the calculation of the correlation coefficient between repeat number and PIC values.

RESULTS AND DISCUSSION

Of the 349 primer pairs tested, 149 (42.7%) showed positive PCR amplification in all three accessions. From those, 77 (51.7%) were polymorphic in two or three accessions (Table 1). For each polymorphic locus, the number of observed alleles per locus ranged from two to six with an average of 2.33, and their PIC values ranged from 0.35 to 0.81. The primer pair showing the largest number of alleles was CMMS4-3, showing polymorphisms in all three accessions. Of the amplified primer pairs, the number of polymorphic primer pairs between two accessions, namely, PI 147065 and PI 320052, PI 147065 and PI 364475, and PI 320052 and PI 364475 was 41 (27.5%), 64 (43.0%), and 70 (47.0%), respectively (Table 1). The remaining 206 primer pairs did not amplify in all three accessions (Table 2).

Fukino et al. (2007) reported that the correlation coefficient between repeat number and PIC values in melon and simple sequence repeat (SSR) markers with ten or more motif repeats are more efficient for detecting polymorphisms. However, in *Brassica* and melon, no correlation has been reported between the degree of polymorphism and the number of repeats (Danin-Poleg et al., 2001; Suwabe et al., 2002). In the present study, the correlation coefficient between the repeat number and PIC was low (0.11). Therefore, it was not necessary to consider the repeat number when applying this technique to gherkins.

In Cucurbitaceae, some DNA markers are transferable to other species. Danin-Poleg et al. (2000) and Fukino et al. (2007) reported the conservation of microsatellite loci between melon and cucumber. Furthermore, Chiba et al. (2003) reported the application of melon microsatellite markers to 9 species of Cucurbitaceae, cucumber, pumpkin (*Cucurbita moschata* L., *C. maxima* L., *Cucurbita pepo* L.), watermelon (*Citrullus lanatus* [Thunb.] Matsum. and Nakai), bottle gourd (*Lagenaria siceraria* [Mol.] Standl.), ash gourd (*Benincasa hispida* [Thunb.] Cogn.), bitter melon (*Momordica charantia* L.), and snake gourd (*Trichosanthes cucumeroides* [Ser.] Maxim.), many of which were applicable to bitter melon, cucumber, and pumpkin. However, no cross-species

| Marker name ¹ | PCR product (bp) | | | Number of all-l | Dahmann hiam2 | |
|--------------------------|------------------|-----------|-----------|-------------------|---------------------------|-----------|
| | PI 147065 | PI 320052 | PI 364475 | Number of alleles | Polymorphism ² | PIC value |
| CMACC146 | 124/124 | 124/124 | 126/126 | 2 | b,c | 0.35 |
| CMAGN52 | 108/116 | 108/116 | 106/118 | 4 | b,c | 0.67 |
| CMAGN68 | 161/161 | 163/163 | 163/163 | 2 | a,b | 0.35 |
| CMAGN73 | 124/124 | 124/124 | 122/122 | 2 | b,c | 0.35 |
| CMAT35 | 111/111 | 111/111 | 111/111 | 1 | | 0 |
| CMATN22 | 155/155 | 155/155 | 155/155 | 1 | | 0 |
| CMATN90 | 126/126 | 126/126 | 126/126 | 1 | | 0 |
| CMBR1 | 127/127 | 127/127 | 115/115 | 2 | b,c | 0.35 |
| CMBR3 | 176/176 | 180/180 | 180/180 | 2 | a,b | 0.35 |
| CMBR8 | 102/102 | 102/102 | 102/102 | 1 | | 0 |
| CMBR13 | 188/188 | 188/188 | 188/188 | 1 | | 0 |
| CMBR14 | 111/115 | 111/115 | 113/117 | 4 | b,c | 0.67 |
| CMBR15 | 160/160 | 160/160 | 183/183 | 2 | b,c | 0.35 |
| CMBR21 | 174/179 | 174/179 | 174/179 | 2 | | 0.38 |
| CMBR23 | 138/138 | 138/138 | 126/126 | 2 | b,c | 0.35 |
| CMBR26 | 100/100 | 100/100 | 100/100 | 1 | | 0 |
| CMBR31 | 171/171 | 304/304 | 327/327 | 3 | a,b,c | 0.59 |
| CMBR34 | 147/147 | 149/149 | 147/147 | 2 | a,c | 0.35 |
| CMBR39 | 133/133 | 133/133 | 133/133 | 1 | | 0 |
| CMBR41 | 125/125 | 125/125 | 125/125 | 1 | | 0 |
| CMBR42 | 95/95 | 95/95 | 95/95 | 1 | | 0 |
| CMBR44 | 124/124 | 124/124 | 122/122 | 2 | b,c | 0.35 |
| CMBR49 | 132/132 | 130/130 | 132/132 | 2 | a,c | 0.35 |
| CMBR54 | 102/102 | 102/102 | 104/104 | 2 | b,c | 0.35 |
| CMBR56 | 134/134 | 134/134 | 124/124 | 2 | b,c | 0.35 |
| CMBR61 | 132/132 | 132/132 | 130/130 | 2 | b,c | 0.35 |
| CMBR64 | 124/124 | 124/124 | 124/124 | 1 | | 0 |
| CMBR69 | 124/124 | 110/110 | 114/114 | 3 | a,b,c | 0.59 |
| CMBR70 | 132/132 | 132/132 | 132/132 | 1 | | 0 |
| CMBR71 | 95/95 | 95/95 | 85/85 | 2 | b,c | 0.35 |
| CMBR83 | 107/107 | 107/107 | 107/107 | 1 | | 0 |
| CMBR89 | 131/131 | 131/131 | 129/129 | 2 | b,c | 0.35 |
| CMBR91 | 118/118 | 118/118 | 118/118 | 1 | | 0 |
| CMBR100 | 114/114 | 114/114 | 104/104 | 2 | b,c | 0.35 |
| CMBR109 | 116/116 | 116/116 | 116/116 | 1 | | 0 |
| CMBR111 | 106/106 | 106/106 | 106/106 | 1 | | 0 |
| CMBR116 | 222/222 | 216/216 | 212/212 | 3 | a,b,c | 0.59 |
| CMBR124 | 166/166 | 166/166 | 166/166 | 1 | | 0 |
| CMBR134 | 236/236 | 236/236 | 206/206 | 2 | b,c | 0.35 |
| CMBR135 | 124/124 | 140/140 | 140/140 | 2 | a,b | 0.35 |
| CMBR153 | 168/168 | 168/168 | 168/168 | 1 | | 0 |
| CMCCA145 | 131/131 | 137/137 | 137/137 | 2 | a,b | 0.35 |
| CMCT160a | 89/89 | 89/89 | 89/89 | 1 | | 0 |
| CMCT44 | 87/87 | 87/87 | 85/85 | 2 | b,c | 0.35 |
| CMCTN2 | 164/164 | 164/164 | 164/164 | 1 | | 0 |
| CMCTN7 | 110/110 | 124/124 | 104/104 | 3 | a,b,c | 0.59 |
| CMCTN86 | 167/167 | 174/174 | 174/174 | 2 | a,b | 0.35 |
| CMCTT144 | 181/181 | 181/181 | 184/184 | 2 | b,c | 0.35 |
| CMGA104 | 112/112 | 114/114 | 110/110 | 3 | a,b,c | 0.59 |
| CMGA165 | 117/117 | 113/113 | 115/115 | 3 | a,b,c | 0.59 |
| CMGA172 | 124/124 | 122/122 | 124/124 | 2 | a,c | 0.35 |
| CMGAN3 | 203/203 | 197/197 | 199/199 | 3 | a,b,c | 0.59 |

Table 1. Microsatellite markers successful amplified sizes of PCR products and polymorphism in three accessions of gherkin.

| Tabl | е | 1. | Cont. |
|------|---|----|-------|
| | | | |

| CMGAN12 | 148/148 | 148/148 | 146/146 | 2 | b,c | 0.35 |
|-----------|---------|---------|---------|---|-------|------|
| CMGAN21 | 133/133 | 133/133 | 133/133 | 1 | | 0 |
| CMGAN25 | 169/169 | 169/169 | 169/169 | 1 | | 0 |
| CMGT108 | 170/170 | 170/170 | 170/170 | 1 | | 0 |
| CMMS3-2 | 427/427 | 427/427 | 427/427 | 1 | | 0 |
| CMMS4-3 | 158/164 | 161/167 | 156/162 | 6 | a,b,c | 0.81 |
| CMMS33-1 | 372/372 | 374/374 | 379/379 | 3 | a,b,c | 0.59 |
| CMMS33-2 | 282/282 | 282/282 | 282/282 | 1 | | 0 |
| CMMS34-6 | 138/138 | 138/138 | 138/138 | 1 | | 0 |
| CMMS34-8 | 181/181 | 101/101 | 151/151 | 3 | a,b,c | 0.59 |
| CMN01_01 | 180/180 | 180/180 | 180/180 | 1 | | 0 |
| CMN01_02 | 235/235 | 235/235 | 233/233 | 2 | b,c | 0.35 |
| CMN01_35b | 123/123 | 123/123 | 123/123 | 1 | | 0 |
| CMN01_54 | 208/208 | 208/208 | 208/208 | 1 | | 0 |
| CMN01_55 | 187/187 | 187/187 | 187/187 | 1 | | 0 |
| CMN01_74 | 188/188 | 188/188 | 188/188 | 1 | | 0 |
| CMN01_88 | 112/112 | 112/112 | 118/118 | 2 | b,c | 0.35 |
| CMN04_03 | 182/182 | 182/182 | 184/184 | 2 | b,c | 0.35 |
| CMN04_09 | 269/269 | 269/269 | 269/269 | 1 | · | 0 |
| CMN04 16 | 157/157 | 157/157 | 157/157 | 1 | | 0 |
| CMN04_21 | 190/190 | 190/190 | 188/188 | 2 | b,c | 0.35 |
| CMN04_27 | 304/304 | 319/319 | 307/307 | 3 | a,b,c | 0.59 |
| CMN04_37b | 203/203 | 211/211 | 203/203 | 2 | a,c | 0.35 |
| CMN04_67 | 114/114 | 114/114 | 110/110 | 2 | b,c | 0.35 |
| CMN04_89 | 215/215 | 215/215 | 215/215 | 1 | | 0 |
| CMN05_08 | 237/237 | 237/237 | 245/245 | 2 | b,c | 0.35 |
| CMN05_13 | 235/235 | 235/235 | 235/235 | 1 | | 0 |
| CMN05_17 | 250/250 | 250/250 | 250/250 | 1 | | 0 |
| CMN05_60 | 170/170 | 170/170 | 170/170 | 1 | | 0 |
| CMN05_69 | 146/146 | 144/144 | 148/148 | 3 | a,b,c | 0.59 |
| CMN05_73 | 155/155 | 153/153 | 155/155 | 2 | a,c | 0.35 |
| CMN05_75 | 180/180 | 180/180 | 180/180 | 1 | | 0 |
| CMN05_77 | 235/235 | 235/235 | 233/233 | 2 | b,c | 0.35 |
| CMN05_79 | 200/200 | 200/200 | 200/200 | 1 | | 0 |
| CMN05_87 | 168/168 | 168/168 | 168/168 | 1 | | 0 |
| CMN05_89 | 239/239 | 239/239 | 239/239 | 1 | | 0 |
| CMN06_19 | 132/132 | 132/132 | 130/130 | 2 | b,c | 0.35 |
| CMN06_41A | 124/124 | 124/124 | 124/124 | 1 | | 0 |
| CMN06_49 | 181/181 | 181/181 | 181/181 | 1 | | 0 |
| CMN06_60B | 125/125 | 125/125 | 113/113 | 2 | b,c | 0.35 |
| CMN07_19 | 102/102 | 102/102 | 102/102 | 1 | | 0 |
| CMN07_32 | 250/250 | 250/250 | 254/254 | 2 | b,c | 0.35 |
| CMN07_95 | 206/206 | 206/206 | 206/206 | 1 | | 0 |
| CMN09_22 | 102/102 | 102/102 | 102/102 | 1 | | 0 |
| CMN21_25 | 211/211 | 211/211 | 213/213 | 2 | b,c | 0.35 |
| CMN21_29 | 252/252 | 252/252 | 252/252 | 1 | | 0 |
| CMN21_33 | 233/233 | 233/233 | 240/240 | 2 | b,c | 0.35 |
| CMN21_42 | 223/223 | 217/217 | 212/212 | 3 | a,b,c | 0.59 |
| CMN21_55 | 166/166 | 166/166 | 166/166 | 1 | | 0 |
| CMN21_85 | 206/206 | 206/206 | 206/206 | 1 | | 0 |
| CMN21_87 | 219/219 | 219/219 | 219/219 | 1 | | 0 |
| CMN21_88 | 265/265 | 265/265 | 255/255 | 2 | b,c | 0.35 |
| | | | | | | |

Table 1. Cont.

| CMN22_09 | 177/177 | 177/177 | 177/177 | 1 | | 0 |
|-----------|---------|---------|---------|---|-------|------|
| CMN22_11 | 115/115 | 115/115 | 103/103 | 2 | b,c | 0.35 |
| CMN22_85 | 207/207 | 207/207 | 213/213 | 2 | b,c | 0.35 |
| CMN23_01 | 281/281 | 281/281 | 281/281 | 1 | | 0 |
| CMN23_06 | 271/271 | 271/271 | 271/271 | 1 | | 0 |
| CMN23_25 | 168/168 | 168/168 | 161/161 | 2 | b,c | 0.35 |
| CMN23_42 | 125/125 | 125/125 | 127/127 | 2 | b,c | 0.35 |
| CMN23_48 | 173/173 | 167/167 | 173/173 | 2 | a,c | 0.35 |
| CMN53_05 | 227/227 | 227/227 | 227/227 | 1 | | 0 |
| CMN53_10 | 165/165 | 165/165 | 165/165 | 1 | | 0 |
| CMN53_28 | 113/113 | 113/113 | 113/113 | 1 | | 0 |
| CMN53_44 | 105/105 | 107/107 | 107/107 | 2 | a,b | 0.35 |
| CMN53_46 | 248/248 | 243/243 | 248/248 | 2 | a,c | 0.35 |
| CMN53_68A | 261/261 | 261/261 | 261/261 | 1 | | 0 |
| CMN53_72A | 107/107 | 111/111 | 107/107 | 2 | a,c | 0.35 |
| CMN61_13 | 156/156 | 160/160 | 156/156 | 2 | a,c | 0.35 |
| CMN61_14 | 172/172 | 172/172 | 172/172 | 1 | | 0 |
| CMN61 88 | 125/125 | 125/125 | 125/125 | 1 | | 0 |
| CMN62_03 | 164/164 | 164/164 | 164/164 | 1 | | 0 |
| CMN62 21 | 357/357 | 357/357 | 357/357 | 1 | | 0 |
| CMN62_40 | 180/180 | 180/180 | 180/180 | 1 | | 0 |
| CMN62_95 | 97/97 | 97/97 | 97/97 | 1 | | 0 |
| CSTA50 | 165/165 | 167/167 | 165/165 | 2 | a,c | 0.35 |
| CMTAA166 | 173/173 | 166/166 | 173/173 | 2 | a,c | 0.35 |
| CMTAAN100 | 181/181 | 174/174 | 181/181 | 2 | a,c | 0.35 |
| CMTC13 | 182/182 | 182/182 | 182/182 | 1 | | 0 |
| CMTC158 | 176/176 | 176/176 | 176/176 | 1 | | 0 |
| CMTC168 | 183/183 | 183/183 | 183/183 | 1 | | 0 |
| CMTC51 | 261/261 | 258/258 | 261/261 | 2 | a.c | 0.35 |
| CMTCC813 | 136/136 | 136/136 | 136/136 | 1 | | 0 |
| CMTCN1 | 122/122 | 122/122 | 150/150 | 2 | b,c | 0.35 |
| CMTCN30 | 192/192 | 192/192 | 192/192 | 1 | | 0 |
| CMTCN56 | 106/106 | 106/106 | 114/114 | 2 | b,c | 0.35 |
| CMTCN67 | 135/135 | 139/139 | 135/135 | 2 | a,c | 0.35 |
| CSLHCPA | 218/218 | 220/220 | 212/212 | 3 | a,b,c | 0.59 |
| CSTCC813 | 130/130 | 135/135 | 135/135 | 2 | a,b | 0.35 |
| SSR184 | 297/297 | 291/291 | 293/293 | 3 | a,b,c | 0.59 |
| SSR303 | 355/355 | 348/348 | 353/353 | 3 | a,b,c | 0.59 |
| TJ3 | 165/165 | 167/167 | 159/159 | 3 | a,b,c | 0.59 |
| TJ10 | 118/118 | 136/136 | 138/138 | 3 | a,b,c | 0.59 |
| TJ27 | 174/174 | 176/176 | 176/176 | 2 | a,b | 0.35 |
| TJ29 | 126/126 | 126/126 | 126/126 | 1 | , | 0 |
| TJ30 | 162/162 | 162/162 | 162/162 | 1 | | 0 |
| TJ31 | 190/190 | 194/194 | 192/192 | 3 | a.b.c | 0.59 |
| TJ33 | 181/181 | 181/181 | 181/181 | 1 | | 0 |

¹References of primer sequences; 'CMMS': Chiba et al. (2003); 'SSR': Joobeur et al. (2004); 'CMBR': Ritschel et al. (2004); 'CMAGN', 'CMATN', 'CMCTN', 'CMCAN', 'CMTCN', and 'TJ': Gonzalo et al. (2005); 'CMN': Fukino et al. (2007); the others: Danin-Poleg et al. (2001). ²'a', 'b', and 'c' indicates polymorphism between PI 147065 and PI 320052, PI 147065 and PI 364475, and PI 320052 and PI 364475, respectively.

application has been reported, and only few DNA markers have been found in gherkin.

In this study, 149 microsatellite primer pairs were successfully amplified, and 77 primer pairs showed

| Marker name ¹ | | | | | | |
|--------------------------|---------|------------|-----------|-----------|-----------|-----------|
| CMAGN32 | CMBR136 | CMBR82 | CMMS22-2 | CMN06_66 | CMN22_15 | CMN61_90 |
| CMAGN33 | CMBR137 | CMBR84 | CMMS24-3 | CMN06_84 | CMN22_16 | CMN62_05 |
| CMAGN61 | CMBR140 | CMBR88 | CMMS30-3 | CMN07_05 | CMN22_17 | CMN62_08 |
| CMAGN68 | CMBR148 | CMBR9 | CMMS33-2 | CMN07_46 | CMN22_22 | CMN62_41 |
| CMAGN75 | CMBR149 | CMBR90 | CMMS34-8 | CMN07_54 | CMN22_23 | CMN62_74 |
| CMAGN79 | CMBR150 | CMBR92 | CMMS35-1 | CMN07_57 | CMN22_27 | CMN62_89 |
| CMAT141 | CMBR16 | CMBR93 | CMMS35-3 | CMN07_70 | CMN22_44 | CMTA134a |
| CMATN22 | CMBR17 | CMBR94 | CMMS35-4 | CMN08_03B | CMN22_45 | CMTA134b |
| CMBR6 | CMBR18 | CMBR95 | CMMS36-2 | CMN08_04 | CMN22_54 | CMTA170a |
| CMBR10 | CMBR19 | CMBR97 | CMN01_03 | CMN08_22 | CMN22_93 | CMTA170b |
| CMBR101 | CMBR30 | CMCT160a+b | CMN01_07 | CMN08_40 | CMN23_15 | CMTC163 |
| CMBR104 | CMBR33 | CMCT58 | CMN01_15 | CMN08_77 | CMN23_43 | CMTC47 |
| CMBR105 | CMBR35 | CMCTN38 | CMN01_34 | CMN08_79 | CMN23_64 | CMTCN14 |
| CMBR107 | CMBR40 | CMCTN4 | CMN01_38 | CMN08_90 | CMN23_79 | CMTCN18 |
| CMBR110 | CMBR43 | CMCTN5 | CMN01_48 | CMN21_03 | CMN53_19 | CMTCN35 |
| CMBR112 | CMBR45 | CMCTN53 | CMN01_83 | CMN21_04 | CMN53_36 | CMTCN50 |
| CMBR113 | CMBR47 | CMCTN65 | CMN01_86a | CMN21_06 | CMN53_40 | CMTCN6 |
| CMBR114 | CMBR48 | CMGA104 | CMN04_01 | CMN21_09 | CMN53_43 | CMTCN8 |
| CMBR115 | CMBR50 | CMGAN24 | CMN04_03 | CMN21_16 | CMN54_48 | CMTCN9 |
| CMBR117 | CMBR51 | CMGAN80 | CMN04_10 | CMN21_17 | CMN61_15 | CSCCT571 |
| CMBR119 | CMBR53 | CMMS1-3 | CMN04_19 | CMN21_34 | CMN61_27 | CSCTTT15b |
| CMBR121 | CMBR60 | CMMS1-7 | CMN04_35 | CMN21_37 | CMN61_35 | SSR411 |
| CMBR123 | CMBR63 | CMMS2-3 | CMN04_40 | CMN21_41 | CMN61_40A | TJ26 |
| CMBR125 | CMBR66 | CMMS3-1 | CMN04_66 | CMN21_59 | CMN61_44 | TJ30 |
| CMBR126 | CMBR67 | CMMS4 | CMN04_79 | CMN21_62 | CMN61_61 | |
| CMBR127 | CMBR68 | CMMS4-1 | CMN05_82 | CMN21_67 | CMN61_63 | |
| CMBR129 | CMBR75 | CMMS11-3 | CMN06_08 | CMN21_74 | CMN61_64 | |
| CMBR130 | CMBR77 | CMMS12-3 | CMN06_25 | CMN21_77 | CMN61_65 | |
| CMBR131 | CMBR79 | CMMS12-4 | CMN06_57 | CMN21_80 | CMN61_70B | |
| CMBR132 | CMBR81 | CMMS15-4 | CMN06_62 | CMN21_82 | CMN61_81 | |

Table 2. Name of unsuccessful amplified microsatellite markers in three accessions of gherkin.

¹References of primer sequences; 'CMMS': Chiba et al. (2003); 'SSR': Joobeur et al. (2004); 'CMBR': Ritschel et al. (2004); 'CMAGN', 'CMATN', 'CMCTN', 'CMGAN', 'CMTCN' and 'TJ': Gonzalo et al. (2005); 'CMN': Fukino et al. (2007); the others: Danin-Poleg et al. (2001).

polymorphisms in gherkin. Specifically, polymorphisms were observed among PI 320052 and PI 364475 (Table 1). In our previous study, PI 320052 was resistance and PI 364475 was susceptible to *Fusarium oxysporum* f. sp. *melonis* race 1,2y (Matsumoto et al., 2011). Thus, these polymorphic microsatellite primer pairs could be useful for the genetic analysis of resistance. Furthermore, they could also be used as landmarks in linkage studies, for investigating genome structure, and in evolutionary ecology of Cucurbitaceae.

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