RESEARCH NOTE

A single recessive gene controls fragrance in cucumber (*Cucumis sativus* L.)

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[Pramnoi P., Somta P., Chankaew S., Juwattanasomran R. and Srinives P. 2013 A single recessive gene controls fragrance in cucumber (*Cucumis sativus* L.). *J. Genet*. **92**, 147–149]

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most widely cultivated vegetables. It belongs to the family *Cucurbitaceae*. Cucumber fruits are consumed as a vegetable fresh or cooked or in pickled form. Over 95% of the cucumber production is in Asia (FAO 2011 FAOSTAT, available at http://faostat3.fao.org/home/index.html). Fruit quality traits of cucumber include fruit colour, spine colour, stripes, fruit size and firmness. Thailand is part of the centre of diversity of cucumber, where *C. sativus* var. *hardwickii* and primitive cultivars are found (de Wilde and Duyfjes 2010), including cultivars with pleasant fragrance. Leaves and fruits of fragrant cucumbers show pandan-like (*Pandanus amaryllifolius* L.) fragrance, which is the same fragrance possessed by aromatic rice (*Oryza sativa* L.), aromatic soybean (*Glycine max* (L.) Merr.) and aromatic sorghum (*Sorghum bicolor* (L.) Moench).

The fragrance in these plants is principally due to the presence of the volatile chemical compound, 2-acetyl-1-pyrroline (2AP) (Buttery *et al.* 1983a, b; Fushimi and Masuda 2001). Genetic studies in rice (Sood and Siddiq 1978), soybean (AVRDC 2003) and sorghum (Murty *et al.* 1982) revealed that the fragrance is controlled by a single recessive gene. In rice and soybean, researchers have successfully identified that mutation(s) in betaine aldehyde dehydrogenase 2 (*BADH2*) gene, causing null function of protein BADH2, is responsible for the fragrance (Bradbury *et al.* 2005; Juwattanasomran *et al.* 2011, 2012).

In this study, for the first time we report the genetics of fragrance in cucumber. The objective of this study was to determine the mode of inheritance of the fragrance.

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Keywords. fragrance; aroma; cucumber; *Cucumis sativus*.

Materials and methods

Plant materials

Two cucumber accessions, PK2011T202 (PKT) and 301176 (301), were used. PKT is a local cultivar from Thailand possessing pandan-like fragrance, while 301 is an inbred line from Clover Seed Company, Hong Kong, possessing no fragrance. They were crossed to develop F_1 (PKT × 301), F_2, BC_1P_1 (PKT × (PKT × 301)) and BC_1P_2 (301 × (PKT × 301)) populations. Before hybridization, presence or absence of fragrance in PKT and 301 was confirmed. The parents, F_1, F_2, BC_1P_1 and BC_1P_2 populations were grown in an experimental field of Kasetsart University, Thailand, during April to June 2012. F_2, BC_1P_1 and BC_1P_2 populations comprised 128, 108 and 86 plants, respectively. The reciprocal F_1 crosses (PKT × 301 and 301 × PKT) were also compared in the same trail. Leaves and fruits produced from such generations were used to evaluate the fragrance.

Mode of inheritance was determined using the F_2 population, and was confirmed by the BC_1P_1 and BC_1P_2 populations.

Evaluation of fragrance

Leaves and fruits of individual plants were used to determine the fragrance. Thirty days after planting, young leaves from each plant of the parental, F_1, F_2, BC_1P_1 and BC_1P_2 populations were subjected to organoleptic test using KOH method as described by Sood and Siddiq (1978). About 0.8 to 1 g of leaves were cut into pieces and placed in a 15-mL tube. Ten mL of 1.7% KOH was added, and the tube was capped and kept for 10 min at 37°C. It was then opened and fragrance was evaluated by smelling. The fragrance was scored as 1 for no fragrance or 2 for fragrance.

For evaluation of fragrance in fruits only one fruit was determined per plant. Fifteen to twenty days after setting, the fruits were evaluated in the morning (8.30 to 9.30 am). Each fruit was smelled directly by the first author. Fragrance was scored the same way as described above for leaves. In addition to self-pollinated fruits set on the parental, F_1, F_2, BC_1P_1 and BC_1P_2 population plants, cross-pollinated fruits (PKT202 × 301 and 301 × PKT) set on the parental plants were also evaluated to determine if the xenia effect (Denney 1992) affects fragrance. Xenia effect is an immediate effect of pollen parent on maternal tissues, i.e. size, shape, colour, developmental timing, and chemical composition of seeds and fruits in which fruit or seed set on maternal plant (parent) readily exhibits the same phenotype as the pollen parent (Denney 1992). A common example is when a sweet corn cob is pollinated by field corn pollens, endosperm of the pollinated seeds will become field corn type on the sweet corn plant.

Data analysis

Chi-square test (Mather 1951) was used to determine goodness of fit to 3:1 and 1:1 ratios of no fragrance to fragrance in the F_2 and BC_1P_1 populations, respectively. The χ^2 test was performed using the R program v. 2.10.0 (R Development Core Team 2010).

Results

Leaves and fruits of PKT were fragrant, while neither leaves nor fruits of 301 were fragrant. The fruits set on PKT by pollen from 301 showed fragrance as strong as PKT, while the fruits produced on 301 by pollen from PKT had no fragrance. These indicate that there is no xenia effect for fragrance in cucumber. However, leaves and fruits of all F_1 plants possess no fragrance (table 1), suggesting that fragrance is a recessive trait.

In the F_2 population, fragrance scores in leaves and fruits were consistent (data not shown). F_2 plants that possessed fragrance in leaves also possessed fragrance in fruits. Fragrance in the F_2 population segregated in a ratio of 3 no fragrance to 1 fragrance (table 1), indicating that fragrance is controlled by a single gene and confirming the recessiveness of the fragrance.

The mode of inheritance in the F_2 population was confirmed using backcross populations. Progenies derived from the backcross to the fragrant parent segregated into 1 fragrance and 1 no fragrance (table 1), again confirming a single recessive gene conferring the fragrance. All progenies developed from the backcross to the nonfragrant parent showed no fragrance (table 1), further indicating that the fragrance trait is recessive.

Discussion

Fragrance in crops is a special and value-addition trait. Cultivars with fragrance command higher demand and price than cultivars without fragrance. In economic crops such as rice, breeding for fragrant grain quality is a major objective in breeding programmes worldwide. This is due to the wide availability of the fragrance germplasm in rice. In contrast, although cucumber is an important crop, fragrance germplasm in cucumber is very rare. The germplasm from Thailand reported in this study enables breeding for fragrance cultivars in this crop for consumers. Fragrance in crop plants can be considered as a trait related to domestication, thus this implies that cucumber may have long been domesticated in Thailand. More exploration and collection for cucumber germplasm and study of its genetic diversity can lead to better understanding of cucumber domestication in Thailand.

Previous studies in rice (Sood and Siddiq 1978), soybean (AVRDC 2003) and sorghum (Murty *et al.* 1982) showed that fragrance is a recessive trait controlled by a single gene. In this study, segregation in the F_2 and backcross populations also revealed that the fragrance in cucumber is recessive and is governed by a single gene. This is the first report

Table 1. Chi-square tests of segregation of fragrant and nonfragrant plants in F_1, F_2, BC_1P_1 and BC_1P_2 populations derived from the cross between the fragrant cucumber PK2011T202 (PKT) and the nonfragrant cucumber 301176 (301).

Organ examined	Parent/population	No. of plants tested	Ratio fragrant plants to nonfragrant plants	χ^2 (probability)
Leaf	PKT	10	10:0	ND
	301	4	0:4	ND
	F_1 (PKT × 301)	17	0:17	ND
	F_2 (PKT × 301)	128	38:90	1.500 (0.221)
	BC_1P_1 (PKT × (PKT × 301))	108	52:56	0.148 (0.700)
	BC_1P_2 (301 × (PKT × 301))	86	0:86	ND
Fruit	PKT	10	10:0	ND
	301	4	0:4	ND
	F_1 (PKT × 301)	17	0:17	ND
	F_2 (PKT × 301)	107	31:76	0.900 (0.343)
	BC_1P_1 [PKT × (PKT × 301)]	91	43:48	0.275 (0.600)

The χ^2 test was performed for goodness of fit against 3:1 and 1:1 ratios for F_2 and BC_1P_1 populations, respectively; ND, not determined.

on genetics of fragrance in cucumber. We propose the symbol *fgr* for the fragrance allele in cucumber. Mutation(s) in the coding sequence of *BADH2* has been shown to be responsible for fragrance in rice (Kovach *et al.* 2009) and soybean (Juwattanasomran *et al.* 2011). It is possible that fragrance in cucumber is also due to a loss-of-function mutation in *BADH2* gene. The recent availability of a dense genetic linkage map and whole genome sequence of cucumber (Huang *et al.* 2009; Ren *et al.* 2009) enables fine mapping and identification of candidate gene(s) for fragrance.

Single-gene control of fragrance in cucumber together with the previous findings in rice, vegetable soybean and sorghum suggest that the pandan-like fragrance in food crops is a simple trait. However, its recessiveness makes breeding for fragrant cultivars difficult. Since most of the current commercial cucumber cultivars are single-cross F_1 hybrids, the *fgr* allele must be fixed in both parental inbred lines to develop such a hybrid. For each backcross generation, an additional season is necessary, starting from BC_2, to self-pollinate the backcross progenies to identify the plants with *fgr/fgr* genotype for further backcrossing. To circumvent this difficulty, marker-assisted selection (MAS) is an alternative for breeding fragrant cucumber cultivars/lines. MAS for fragrance has been applied successfully to developed fragrant rice lines (Jantaboon *et al.* 2011).

Xenia effect is an immediate effect of pollen parent on maternal tissues, i.e. size, shape, colour, developmental timing, and chemical composition of seeds and fruits in which fruit or seed set on maternal plant (parent) readily exhibits the same phenotype as the pollen parent (Denney 1992). A common example is when a sweet corn cob is pollinated by field corn pollens, endosperm of the pollinated seeds will become field corn type on the sweet corn plant. In this study, we have shown that there is no xenia effect for fragrance in cucumber. On one hand, the absence of xenia effect is an advantage for growers of fragrant cucumber because fruits from flowers pollinated by pollen with *Fgr* allele from adjacent plots are still fragrant. On the other hand, it is a disadvantage for unaware farmers who produce commercial seeds of fragrant cucumber using own-saved seeds. Fruits set from cross pollination by *Fgr* allele are still fragrant but contain seeds of genotype *Fgr/fgr*. Cucumber plants grown from those seeds will set nonfragrant fruits.

Acknowledgements

This research was co-funded by the Thailand Research Fund, and Kasetsart University, Thailand to P. Somta (grant RSA5580057). We also acknowledge support from Graduate School of Kasetsart University to P. Pramnoi.

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Received 13 November 2012; accepted 22 January 2013
Published on the Web: 10 April 2013