トウガラシにおける遺伝的変換 (3)

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HEREDITARY CHANGES IN *CAPSICUM ANNUUM* L. III. INDUCED BY DNA TREATMENT[1)]

SABURO NAWA, MASA-AKI YAMADA AND YASUO OHTA

National Institute of Genetics, Misima, 411
Kihara Institute for Biological Research, Misima 411

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In the preceding papers we presented evidence of hereditary changes at gene level induced by ordinary and virus-inoculated graftings in red peppers, *Capsicum annuum* L. (Ohta and Chuong 1975a, b). A hypothesis has been proposed that the changes could be induced by DNA translocated from the stock to the scion, a kind of transformation or transduction (Ohta 1970). We have already obtained the hereditary changes induced by DNA treatment in insects (Nawa and Yamada 1968, Nawa *et al.* 1971). Thus, the experiment of DNA treatment with red peppers was carried out to examine the possibility of transformation.

MATERIALS AND METHODS

The same materials were used as in the grafting experiments reported in the preceding papers (Ohta and Chuong 1975a, b): cultivars Tochigisantaka (abbreviation T) and Kiiro (K) of red peppers, *Capsicum annuum* L. Three fruit characters were chosen for analysis: fruiting direction as being erect (*up*) vs. pendent (*Up*), fruiting habit or position as fasciculate (*fa*) or clustering vs. non-fasciculate (*Fa*) or non-clustering, and ripe pericarp color as yellow (yc_1) vs. red (YC_1). These characters are Mendelian traits and the four genes are inherited independently (Ohta and Chuong 1975a).

DNA was prepared from young leaves of T (*upup*, *fafa*, *YY*, C_1C_1) or K (*UpUp*, *FaFa*, *yy*, c_1c_1) which had been stored in a freezer according to a modification of the method of Marmur (1961). Frozen leaves were minced with a knife and then crushed with mortar and pestle in a cold solution of 0.14 M-NaCl plus 0.1 M-EDTA, adjusted to pH 8. Sodium dodecylsulfate was added until its final concentration of 2.5%; the mixture was maintained at 60°C for 5 min. and then cooled. 5 N sodium perchlorate was added to the lysate until its final concentration was 1 N; and the suspension was then shaken with an equal volume of chloroform. After centrifuging, the aqueous phase was collected and crude fibrous DNA was precipitated by adding 2 volumes of

1) The abstract of this paper has been presented at the XIII International Congress of Genetics held in August 1973 at Berkeley, California, U.S.A. as both a contributed paper and an exhibit including real herbarial specimen and color pictures of the variants genetically analyzed.

alcohol. The sediment was dissolved in SSC (0.14 M-NaCl plus 0.014 M-trisodium citrate) and deproteinization was completed by two cycles of chloroform washing and reprecipitation with alcohol. Then, the DNA, dissolved in SSC, was treated with RNase and α-amylase, followed by further chloroform deproteinization. The DNA was further purified by treatment with cetyltrimethylammonium bromide (CTAB) (Jones 1963). To the DNA dissolved in 1 M-NaCl was added an aqueous solution of CTAB (3% W/V) until the NaCl concentration was reduced to 0.3 M. The resulting fiber wound on a glass rod was redissolved in 1 M NaCl and then treated with two cycles of chloroform washing and reprecipitation with alcohol. The purified DNA was finally dissolved in 0.01% NaCl. The DNA samples prepared in this way were contaminated with 20—25% RNA and 8—12% polysaccharides. The RNA was not removed by repeated treatment with RNase. In one specimen, the intrinsic viscosity was 93, giving an approximate molecular weight of 2×10^7.

DNA treatment was made in the following two ways: a) After treatment with 1% NaClO solution for 30 min., germinating T seeds were immersed at 25°C for 4 days in the solution of K-DNA extracted from K (100 μg/ml 0.01% NaCl). The DNA solution was renewed every day. They were then transferred to absorbent cotton moistened with the K-DNA solution and kept for two days and then washed with distilled water. After germination, seedlings with cotyledons only were again immersed in the K-DNA solution for 5 hours and then transplanted into pots. The same treatment was applied to K seeds with T-DNA extracted from T. b) Seeds of T, which had been treated with 1% NaClO solution for 30 min., were immersed in the K-DNA for 4 hours and then sown and germinated. Seedlings with 2-3 leaves were cut at the hypocotyl, immersed again in the K-DNA solution for 1 hour, and then grafted as scions onto K-stocks. The same treatment was applied to K seeds with T-DNA.

RESULTS AND DISCUSSION

No unusual phenomenon was observed in the DNA-treated generation. Flowers of these treated individuals were self-pollinated and the next generation was raised for examination. The results are given in Table 1.

In the case of T*(K-DNA) (that is, selfing of T plants treated with K-DNA) one plant out of 393 individuals examined was found to be a variant which carried a trait of donor DNA, non-fasciculate. Other fruit characters remained unchanged. The change is from recessive homozygous to heterozygous or dominant homozygous, *fafa*→*Fa*—. Erect (*upup*), another recessive homozygous trait, has not changed; this evidence rules out the possibility of contamination. In the case of K*(T-DNA) no variant was obtained among 466 individuals examined. The variant rate was one out of 393 or 0.25 per cent for T*(K-DNA) and one out of 859 or 0.12 per cent for both T*(K-DNA) and K*(T-DNA) treatments.

On the other hand, a variant was obtained among 497 individuals in the selfed progeny of T*(K-DNA)/K (selfing of T plants treated with K-DNA and grafted onto K-stocks). This individual possessed fruits of pendent, non-fasciculate, red pericarp

Table 1. Production of variants by treatment with DNA

	Treatment	Treated generation		Selfed progeny	
		No. of individuals examined	No. of variants obtained	No. of individuals examined	No. of variants obtained
a)	T*(K-DNA)	13	0	393	1
	K*(T-DNA)	21	0	466	0
b)	T*(K-DNA)/K	14(12)**	0	497	1
	K*(T-DNA)/T	7(3)**	0	32	0

*) Selfed seeds were obtained from those with asterisk.
**) Figures in parentheses indicate the number of individuals from which selfed seeds were obtained.

and K-shape. In other words, two Mendelian traits had changed in this variant. The changes were *upup*→*Up*—, *fafa*→*Fa*—, and from T-shape to K-shape. It is not clear in this case whether this variant was induced by DNA treatment or by grafting or by both. In the case of K*(T-DNA)/T no change was observed in the selfed progeny, although the number of individuals was very small. The rate of variant was one out of 497 or 0.20 per cent for T*(K-DNA)/K and 0.19 per cent for both T*(K-DNA)/K and K*(T-DNA)/T treatments.

It may be said, therefore, the rate of variants by DNA treatment only and that by DNA treatment plus grafting were almost the same: approximately 0.2 per cent. The overall variant rate was 0.84 per cent by ordinary grafting in the same materials as in the present experiment (Ohta and Chuong 1975a). Therefore, the present rate appears to be rather low; this might be due to the small scale of experiments. No spontaneous variants were obtained in untreated plants (Ohta and Chuong 1975a).

The phenomenon of the uptake of foreign DNA by living cells of higher organisms has been confirmed in several laboratories with different systems. Recently, evidence has been presented in *Arabidopsis* that the foreign DNA can be integrated and replicated in the host cells and that treatment with DNA produces some biological effects transmissible to the progeny (Ledoux *et al.* 1971a, b). Results in favor of transformation induced by DNA have also been reported in petunia (Hess 1969a, b). Thus, it would be expected that the variants obtained in the present experiments are transformants. Moreover, the changes are identical with those by ordinary grafting reported in the preceding paper (Ohta and Chuong 1975a). It may be said, therefore, that the mechanism of graft-induced hereditary changes should be essentially transformation, if DNA could be translocated from the stock to the scion.

SUMMARY

DNA of molecular weight of about 2×10^7 was extracted from red peppers. When seeds of T cultivar (*upup*, *fafa*, *YY*, C_1C_1) were treated with DNA from K cultivar (*UpUp*, *FaFa*, *yy*, c_1c_1), a variant was obtained which shows a dominant character,

non-fasciculate (*Fa*—), of the DNA donor, but maintains another recessive trait, erect (*upup*). Another variant was obtained in which two Mendelian traits changed to those of the DNA donor (*up*→*Up*, *fa*→*Fa*). The changes were exactly the same as those induced by ordinary grafting, suggesting that the mechanism of hereditary changes obtained by grafting could be induced by DNA derived from the stock.

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