Transferring total Genomic DNA of H. tuberosus into the maintenance line as well as the generation performance

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Summary

During the full flowering, self pollination of 7718B line was carried out first, then interval a certain time, stigmas of every disk flower were cut off and transferred DNA (or DNA+JFC, DNA+LSSC) solution of H. tuberous. plant and seed characters of F_0-F_4 generations were evaluated and compared with control. F_0 plant and seed was similar to the control. Plant height, head diameter, number of leaves, day of growth, thousand seeds weight, seed color in F_2 generation had great separation. It showed that DNA solution would effect on the development of B line. F_3 generation plant tended to stable. Identified F_4 breeding lines that some of its resist downy mildew PL_2 and some has early maturity.

Additional Index words: DNA solution, pollination, separation, resistant, maturity.

Since beginning Nineteenth Century planters used hybridization heterosis, from that time hybridization heterosis had been used in lot of crop. Improving yield and quality of crop has been conducting for getting more seed grain and vegetables. However germplasm improvement of cultivated varieties have been applying, following the resistibility and adaptability of varieties gradually decreased as well as disease were serious year to year. For enhancing and compensation genetic base of cultivated varieties experts have been working on the homogenetic wild species and would hope to transfer available characters to cultivated varieties. Present studies objective that utilization total DNA of H. tuberosus improved the characters of 7718B line and developed a excellent line.

material and method

Maintenance line 7718B as female was planted a trial plot in spring in 1992. Selected 5-7 tubers of H. tuberosus were planted in the bowl soil for emergence. The 7718B line was full flowering two weeks ago, using buds of H. tuberosus tubers extracted the total DNA and made up DNA solution(some of it mixed with JFC, some mixed with LSSC) that was placed in the glass bottle.

To select 7718B 60 plants were baged before flowering in the trial plot. Casing bag plants pollinated by self respectively, then 2-3.5 hours interval, stigma in every disk flower was cut and placed DNA solution on the styles. Whereas the DNA

solution entered the Ovary along the way of the pollen tube and participated in an embryogeny as well as endosperm development.

Handling as following:
selected three controls
1. On June 22, 1992. Using normal plant of casing bag in the trial plot conducted self-pollination no cutting stigma as CK_1.
2. On June 24, 1992. Using normal casing bag plants in the trial plot conducted by self-pollination, then three hours later, cut stigma as CK_2
3. On June 24, 1992. Using normal casing bag plants in the trial plot conducted by self pollination, interval three hour twenty minutes, then cut disk flower stigma as CK_3.
Self-pollination had been conducted while every disk flowering reached two-thirds. After pollination and handling, it didn't rain for five hours and recorded numbers and type of handling plants. The control and each handling plants were harvested in Autumn and identified agronomic characters of lines. Selecting F_0 generations were planted in the trial plot next year. Variance of each character of the lines were Observed and recorded continually every year.

results and discussion

F_0 generation seeds between handling plants and the control did not differ in phenotype characters. Total saving material was 19 generations in 1993, among them seeds of five progenies were not emergence, seedlings of some progenies were very little. Growth performer of all phenotype characters of all plants in the trial plot was the same or similar, but seed grains of 46 generation lines(F_1) with self pollination varied very greatly. Seed color in the handling 2 became a nooilseed color (A→E). Ninety percentage seed grain shape of head to compare with the control have a large variance, some of them was a deltoid, numbers were an elliptic.
F_2 generation, it was very segregation period. Plant height, shape of plant and leaves, hull percentage, seed color, thousand seed weight had many different in the progenies among lines and between lines. (table 2)
F_3 generation, sowing seventy four F_2 generations harvested 238 lines. On the basis of F_2 generation, many posterity have trend stable. Major agronomy characters have been the same or similar in F_2 and F_3 generation plant and seed, such as plant height, head diameter, number of leaves, hull color, day of growth. F_4 generation,identification of resistibility to deceases was conducted in a number of F_4 lines, Dj25-273 resisted the downy mildew PL_2. 12 lines have a earlier maturity than the control.
Using total DNA transfer method may create new breeding materials and improve characters of line was developed. This method would enhance genetic basis of sunflower.

Handling table 1

NO.	date	pollitime	interval time	cutting and smearing time	after cutting stigma smearing types
1	on June 22	11:20	2:50	13:50	only smearing DNA solution
2	on June 22	11:30	2:00	13:30	only smearing DNA solution
3	on June 22	11:15	2:55	14:10	only smearing DNA solution
4	on June 22	11:15	2:00	14:00	only smearing DNA solution
5	on June 22	11:00			CK_1 self-pollination only
6	on June 22	11:10	3:10	14:20	only smearing DNA solution
7	on June 23	11:30	3:00	14:30	only smearing ISSC
8	on June 23	11:10	2:30	13:40	only smearing DNA solution
9	on June 23	11:20	3:10	14:30	only smearing ISSC
10	on June 23	11:20	3:10	14:30	only smearing DNA solution
11	on June 23	11:30	2:40	14;10	only smearing DNA solution
12	on June 23	11:15	2:35	13:50	only smearing DNA solution
13	on June 24	11:20	2:50	14;10	smearing DNA+0. 25ISSC
14	on June 24	11:00	2:40	14:40	smearing DNA+0.25JFC
15	on June 24	11:00	2:30	14:30	smearing DNA+0.25JFC
16	on June 24	11:20	2:00	14:00	smearing DNA+0.25JFC
17	on June 24	11:20	3:00	14:20	CK_2 cutting stigma then casing bag
18	on June 24	11:30	3:20	14:50	CK_3 cutting stigma then casing bag
19	on June 24	11:10	2:30	13:40	smearing DNA+0.25 ISSC+0.25JFC

note: JFC-permeable solution, ISSC-buffer solution

table 2 F_2 generation characters separation

handling No.	plant height(cm)	handling No.	head diameter(cm)	handling No.	stem diameter(cm)	handling No.	number of leaves
$control_{17}$	130	$control_{17}$	17.0	$control_{17}$	2.6	$control_{17}$	28
15	166.6	15	22.5	4	2.9	4	31
2	70.0	2	11.9	12	1.8	12	22
range	166.6-70.0	range	22.5-11.9	range	2.9-1.8	range	31-22

handling No.	days of growth	handling No.	husk of percentage (%)	handling No.	thousand seed weight (g)	handling No.	color husk of seed
$control_{17}$	75	$control_{17}$	30	$control_{17}$	7.0	$control_{17}$	A
15	81	4	44	19	12.5	2	E
2	75	4	22	10	2.0	8	E
range	81-75	range	44-22	range	12.5-2.0	range	A-E